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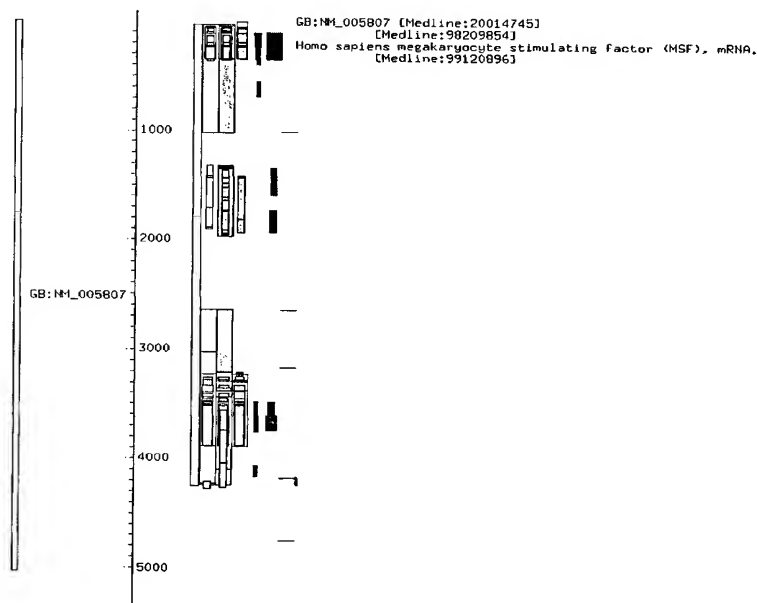
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(54) Title: **NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH OSTEOARTHRITIS**



(57) Abstract: The invention relates to novel polynucleotides associated with human disease, and in particular to osteoarthritis. The invention further relates to polymorphic polynucleotides associated with osteoarthritis. The invention provides methods of determining if a particular polymorphism predisposes an individual to or is associated with the development of osteoarthritis. The invention also provides methods of detecting the presence of one or more polymorphism as an indicator of osteoarthritis, and provides for use of novel polynucleotides of the invention in the development of drugs and in disease treatment.



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## NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH OSTEOARTHRITIS

## TECHNICAL FIELD

The invention relates in general to polymorphisms in genes associated with osteoarthritis and bone remodeling and methods of identifying individuals having a gene containing a polymorphism associated with osteoarthritis. The invention also relates to a method of detecting an increase in susceptibility to a disease in an individual resulting from the presence of a polymorphism or mutation in the gene coding sequence of a osteoarthritis and bone remodeling associated gene.

## BACKGROUND OF THE INVENTION

Single nucleotide substitutions and small unique insertions and deletions are the most frequent form of DNA polymorphism and disease-causing mutation in the human genome. These DNA sequence variations, called single nucleotide polymorphisms (SNPs), have gained popularity and have been proposed as the genetic markers of choice for the study of complex genetic traits (Collins et al. 1997 Science 278: 1580- 1581; Risch and Merikangas 1996 Science 273: 1516-1517). Despite the fact that on average approximately one nucleotide position in every 1000 bases along the human chromosome is estimated to differ between any two copies of the chromosome (Cooper et al. 1985 Human Genetics 69: 201-205; Kwok et al. 1996 Genomics 31: 123-126) developing SNP markers is not easy.

It has been suggested that association studies (such as linkage equilibrium studies) with a set of single nucleotide polymorphism (SNP) markers evenly spaced across the genome at approximately 100 KB intervals would provide the necessary power to detect the small effects of each gene involved in a complex trait (Hauser et al. 1996 Genetic Epidemiology 13: 117-137 in Kwok and Chen 1998 Genetic Engineering 20: 125-134, Plenum Press, New York). Alternatively, one can take a candidate gene approach in performing association studies with the use of a set of gene-associated SNP markers to detect these genetic factors (ibid.).

Nucleotide sequence mutations which occur in a gene or gene family, where the gene or gene family is associated with a given disease, may be the basis for susceptibility to or development of the disease.

Arthritis means "inflammation of a joint" and encompasses more than a hundred diseases. They can affect the joints and other connective tissues such as muscles, tendons, ligaments and protective coverings of internal organs. The major arthritis diseases are as follows:

1. osteoarthritis - non-inflammatory degenerative joint disease characterised by splitting and

fragmentation of the articular cartilage, hypertrophy of the bone and changes in the synovial membrane.

2. rheumatoid arthritis - chronic systemic, relapsing disease primarily of the joints which is marked by inflammatory changes in the synovial membranes and adjacent structures.

3. ankylosing spondylitis - inflammatory disease that affects the joints of the lower back which may lead to fusion of the spine

4. gout - caused by formation of uric acid crystals in the joint, leading to inflammation and severe pain.

Osteoarthritis is the most common type of arthritis. It differs from rheumatoid arthritis in that it is primarily a degeneration of the joint tissue that may be accompanied by an inflammatory reaction (Figure 1). Rheumatoid arthritis is an inflammatory disease first and foremost and inflammation of the synovium is the focal point of the disease.

The initiation and progression of osteoarthritis involves multiple pathogenic mechanisms. An imbalance of chondrocyte-controlled anabolic and catabolic processes results in a progressive degradation of the components of the extracellular matrix of the articular cartilage, associated with secondary inflammatory factors. The primary cause of this is unknown but possibly involves a deficiency of cellular response to normal tissue demand or insufficient cellular response to supernormal demand from mechanical loading or injury. The subsequent repair response could induce elevated levels of anabolic molecules, leading to remodelling of the bone and production of osteophytes (bone outgrowths) characteristic of the disease process.

Prevalence and social cost of osteoarthritis.

With approximately 40 million Americans affected by arthritis and other inflammatory diseases, the cost to the healthcare system is significant. Of these 40 million people, 21 million have osteoarthritis and 2.1 million have rheumatoid arthritis. Osteoarthritis is the most common chronic condition and cause of inactivity in patients older than 65. The disease occurs usually at the beginning of the fifth decade of life, with increasing prevalence and incidence with advancing age (Table 2). The prevalence of arthritis is expected to increase by 57% by the year 2020. In the same time period, arthritis-causing activity limitation will increase 66% to 11.6 million people (Lawrence et al 1998). The primary impact of arthritis in the elderly is decreased physical functioning. This can be due to other health-related problems, such as weight gain, cardiovascular disease, GI distress related to treatment, increased psychological distress, decreased social functioning, increased work disability, and



increased healthcare utilization. The current OA treatment, NSAIDs are responsible for the highest number of hospitalisations of any drug category and cause a significant number of internal gastrointestinal bleeding in the elderly population.

The cost of arthritis in the US (including rheumatoid arthritis, osteoarthritis and all other rheumatic conditions) was shown to be \$64.8 billion in 1992. Of this, direct costs were an estimated \$15.2 billion and indirect costs \$49.6 billion (Yelin and Callahan 1995). A 1997 study showed the cost of care for osteoarthritis as \$543 per patient per year (Lanes et al 1997). The largest component was hospital care, mostly due to admissions for hip or knee replacement. The cost to the healthcare provider is very high due to the prevalence of the illness.

#### Unmet medical needs for OA

Current treatment options for osteoarthritis focus on symptom relief whereas truly disease-modifying agents or methods are lacking. Thus, the basic therapy includes common analgesics, nonsteroidal anti-inflammatory drugs, physical therapy, walking aids, and eventually in severe cases, joint replacement surgery. Perhaps because of the difficulties involved in measuring disease progression existing medications do not address the need to prevent further cartilage degradation.

To develop such drugs the following should be in place:

- Compounds that target appropriate biochemical pathways (e.g. Merck's MMP-3 antagonist)
- Clinical studies must be able to measure disease progression in a cost-effective and safe fashion. This could be either an imaging technique or a biomarker that closely correlates with disease progression.

- Disease progression should be detectable within a reasonable time scale (for example, anti-inflammatory clinical studies use the WOMAC pain scale for a period of 6 weeks to measure improvement due to medication).

- The efficacy of the new drug under development should be observable (using either the imaging or biomarker method of assessment) in a sample size comparable to that of other clinical trials.

How can genetics help? Genetic studies have the potential to detect:

- Novel drug targets in the appropriate pathways.
- Individuals with fast progressing osteoarthritis. This would allow a pharmaceutical company to prove efficacy in a relatively small sample size and in a reasonable period

of time, thus cutting costs.

- Reduce variation from biomarker or imaging patterns. For example, let's assume the following response to medication. Although there is a clear patterns of response to medication, it is not statistically significant because of the large amount of variation in disease progression. Lets now assume that there exists a genetic marker that is able to stratify the measurement of disease progression in this hypothetical study. The variance of the marker of disease progression associated with each genotype is smaller than the overall variance. This can be seen as analogous to stratifying a relevant clinical measure in a study (e.g. lipid levels) by gender or by age group. By pooling together both genders or both age groups the variance is larger. If we were now to stratify the results of the previous hypothetical study by genotype we might observe that the therapeutic efficacy is now statistically significant. By stratifying according to genotype it could then be possible to detect statistically significant efficacy in both groups, while meeting the cost and time needs of the entity developing the drug.

#### 15 Genetic study of osteoarthritis.

Evidence for genetic predisposition to OA.

The nature of the genetic influence in osteoarthritis may involve either a structural defect (that is, collagen), alterations in cartilage or bone metabolism, or a genetic influence on a known risk factor for osteoarthritis such as obesity. Twin studies have show that between 39% and 65% of osteoarthritis in the general population can be attributed to genetic factors (MacGregor and Spector, 1999). Linkage analyses (i.e., common inheritance of affected individuals in the same family) have identified a higher risk ratio for relatives of affected individuals compared to the general population. The power to detect disease-susceptibility loci through linkage analysis using pairs of affected relatives depends on  $I_R$ , the risk ratio for type R relatives compared with population prevalence (Risch 1990). Kellgren et al. (1963) compared expected and observed incidence of osteoarthritis in first-degree relatives of probands with multiple osteoarthritis. Based on their results we have estimated  $I_R$  for nodal and non-nodal osteoarthritis.

Type of OA	$I_R$
Nodal (presence of Heberdeen's nodes)	4.5
Non-nodal	4.75

For comparison, concordance for type 2 diabetes ranges between 2-3, and between 4.5 and 5.5 for rheumatoid arthritis. These figures indicate a high genetic component to OA. If, however, non-nodal and nodal types of OA are mixed together  $I_R$  drops to  $\sim 2.0$  highlighting the importance of careful clinical characterization for genetic studies.

Although it is known that there is a genetic component involved in the etiology of osteoarthritis there is also a need in the art for an improved understanding of the genetic causes of osteoarthritis.

There is also a need in the art for identification of the genes associated with osteoarthritis, and identification of sequence variations in these genes that are associated with osteoarthritis and bone remodeling. The identification of disease related sequence variations in osteoarthritis and bone remodeling associated genes will allow for the development of improved methods of screening for osteoarthritis. These improved screening protocols may be used to identify individuals at high risk for osteoarthritis and in need of preventative treatments.

The identification of disease related sequence variations in osteoarthritis associated genes may facilitate the design of treatment protocols and the identification and design of compounds useful for treatment of osteoarthritis and bone remodeling.

## OBJECTS AND SUMMARY OF THE INVENTION

An object of the present invention is to provide candidate genes associated with osteoarthritis and bone remodeling.

It is another object of the present invention to provide a variant nucleotide in a candidate gene associated with osteoarthritis and bone remodeling.

Another object of the present invention is to provide methods of detecting variant nucleotides in a gene in individuals at risk for osteoarthritis.

Another object of the present invention is to provide methods of determining if a variant nucleotide is associated with a predisposition to osteoarthritis.

Another object of the present invention is to provide candidate genes associated with the osteoarthritis and bone remodeling.

The invention further comprises isolated polynucleotides which contain the single nucleotide polymorphisms selected from the Sequence Listing, or its perfect complement.

The invention further comprises an isolated polynucleotide segment of between 10 and 100 bases of which 10 contiguous bases including a polymorphic site are from a sequence selected from the Sequence Listing, or its perfect complement.

The invention further comprises a probe or target sequence used for genotyping where the

probe or target sequence has at least 10 contiguous bases containing a polymorphic site identified and from a sequence selected from the Sequence Listing, or its perfect complement.

The invention further comprises a method for determining a base occupying a polymorphic site in a nucleic acid comprising obtaining the nucleic acid in a sample from an individual or plurality of individuals and determining a base occupying a polymorphic site in a sequence selected from the group consisting of the Sequence Listing and their perfect complements which occurs in the sample nucleic acid.

### DESCRIPTION OF THE COMPACT DISK-RECORDABLES (CD-R)

CD-R (Copy 1) contains the Sequence Listing formatted in plain ASCII text and Tables 1 and 2. CD-R (Copy 1) is labeled with Identification No. GX-0022P-1.

CD-R (Copy 2) is an exact copy of CD-R (Copy 1). CD-R (Copy 2) is labeled with Identification No. GX-0022-1 P (Copy 2).

CD-R (Copy 3) contains the Computer Readable Form of the Sequence Listing in compliance with 37 C.F.R. §1.821(e), and specified by 37 C.F.R. §1.824. CD-R (Copy 3) is labeled with Identification No. GX-0022-1 P (Copy 3).

The material on CD-R 1, 2 and 3 is incorporated by reference into the specification.

### BRIEF DESCRIPTION OF THE TABLES AND DRAWINGS

These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, appended claims, and accompanying tables and drawings where:

Table 1 presents the genomic or cDNA structure of osteoarthritis candidate gene sequences and the identity and position of polymorphisms which are the subject of the invention. This table has the form wherein:

a. The DNA change given for an allele is not strand specific; it can be on either strand of the DNA molecule.

b. Single Nucleotide Polymorphisms can be recorded as IUPAC ambiguity symbols, as follows:

M	A or C
R	A or G
S	C or G
K	G or T

W     A or T

Y     C or T

c. Other allele types, such as insertions and deletions, are given in the form:    ACA>AA

or     AA>ACA and in such cases the coordinates of the allele include the two invariant

5   flanking bases.

d. DNA sequence names are of the form: XX:IIIII[\_VV], where XX gives the database of origin, as follows:

EM     EMBL

FN     Incyte FL sequence read

10     GB     GenBank

IN     Incyte proprietary sequence

LG     LifeSeq Gold gene template

IIIII gives the sequence ID or accession number for the sequence. In most cases if it is an  
15   accession number it will be followed by \_VV where VV is the sequence version in the EMBL or GenBank database.

e. The overall structure of a record in the patent structure is described as follows. Items in {braces} indicate a field that is filled in. Items in [square brackets] may or may not be present.

These entries define a larger virtual sequence, a "link" composed of real database subsequences.

20   Alleles are annotated onto real sequences, and genomic structure onto the link.

{Locus ID}

[Full name : {full name}]

Link : {link name}

Subsequence    {name} {link start position} {link stop position} {SEQ ID NO}

25   [...]

CDS    {name} {SEQ ID NO}

exon/ORF {link start position} {link stop position}

[...]

[...]

30   Allele   {seq name} {SEQ ID NO} {seq start} {seq stop} {dna change}

source {original SNP data source} {SNP id in that source}

[...]

consequence {CDS name} {CDS SEQ ID NO} {class} [{peptide pos} {peptide

change}}]

[...]

[...]

f. Sources. SNPs may have been noted in one of several sources:

dbSNP The NCBI public dbSNP databank

isSNP In silico SNPs from LifeSeq sequence assembly.

wetSNP Alleles determined by SSCP.

Alleles which have a wetSNP entry are experimentally verified. Alleles which are isSNP and/or dbSNP only are predictions by computer software of where these SNPs map to, and are \*not\* experimentally verified.

g. Consequences

The classes of consequence are as follows:

Silent The allele does not cause a peptide change

Missense The allele causes an amino acid substitution

Frameshift The allele causes a frame shift in the CDS

Intron The allele lies wholly within an intron.

5' The allele lies 5' of the CDS

3' The allele lies 3' of the CDS

Unknown The consequence is undefined - for example the allele straddles an intron/exon boundary.

Silent and Missense consequences also supply details of the amino acid position of the change, and prediction of what the affected amino acid is, and what it is substituted to. There may be multiple consequence lines if the locus contains multiple CDS forms.

h. Sequence and exon positions

Sequence coordinates are always given on the forward strand of the link. Therefore, if a sequence or exon is actually on the reverse strand of the link, its start position will be larger than its stop position.

i. Exon order in CDS definitions

The exons are given in 5' to 3' order. Consequently, reverse strand CDS start from high coordinate numbers downwards.

j. Link object types

Loci may have more than one link object, composed of different DNA sequences. Typically there might be one genomic and one cDNA link object.

Table 2 presents the population frequency of polymorphisms in the candidate genes and summarizes various information from Table 2 relating to the polymorphism.

Figure 1 illustrates the cDNA structure of the locus and relative positions of identified SNPs for megakaryocyte stimulating factor (MSF).

5 Figure 2 illustrates the genomic structure of the locus, exons composing multiple CDS, and relative positions of identified SNPs for megakaryocyte stimulating factor (MSF).

The figures show (from left to right) the real sequences making up the linked genomic structure for the locus, a scale in link coordinates (negative numbers would indicate a view of the reverse strand), one or more CDSs representing the positions of exons, horizontal bars representing  
10 the positions of identified SNPs (alleles) from the various sources, and shaded boxes showing regions targeted for screening by SSCP.

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

15 Before the present compositions and methods are described, it is understood that embodiments of the invention are not limited to the particular machines, instruments, materials, and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the  
20 invention.

As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a nucleic acid probe" includes a plurality of such nucleic acid probes, and a reference to "a gene" is a reference to one or more genes and equivalents thereof known to those skilled in the art, and so forth.

25 Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the  
30 cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with various embodiments of the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

### Definitions

As used herein, "polymorphism" refers to a nucleotide alteration that either predisposes an individual to a disease or is not associated with a disease, which occurs as a result of a substitution, insertion or deletion.

More particularly, a "polymorphism" or "polymorphic variation" may be a nucleic acid sequence variation, as compared to the naturally occurring sequence, resulting from either a nucleotide deletion, an insertion or addition, or a substitution, which is present at a frequency of greater than 1% in a population.

As used herein, "neutral polymorphism" refers to a polymorphism which is present at a frequency of greater than 1% in a population, which does not alter gene function or phenotype, and thus is not associated with a predisposition to or development of a disease.

As used herein "polynucleotide sequence" refers to a sense or antisense nucleic acid sequence comprising RNA, cDNA, genomic DNA, synthetic forms and mixed polymers, that may be chemically or biochemically modified or may contain non-natural or derivatized nucleotide bases.

As used herein "mutation" refers to a variation in the nucleotide sequence of a gene or regulatory sequence as compared to the naturally occurring or normal nucleotide sequence. A mutation may result from the deletion, insertion or substitution of more than one nucleotide (e.g., 2, 3, 4, or more nucleotides) or a single nucleotide change such as a deletion, insertion or substitution. The term "mutation" also encompasses chromosomal rearrangements.

As used herein, "nucleic acid probe" refers to an oligonucleotide, nucleotide or polynucleotide, and fragments and portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single- or double- stranded, which represents the sense or antisense strand. Both terms "nucleic acid probe" and "DNA fragment" refer to a length of polynucleotide, for example, as small as 5 nucleotides, 10, 20, 25, 40, 50, 75, 100, 250, 400, 500 and 1 kb, and as large as 5-10kb.

As used herein, "alteration" refers to a change in either a nucleotide or amino acid sequence, as compared to the naturally occurring sequence, resulting from a deletion, an insertion or addition, or a substitution.

As used herein, "deletion" refers to a change in either nucleotide or amino acid sequence wherein one or more nucleotides or amino acid residues, respectively, are absent.

As used herein, "insertion" or "addition" refers to a change in either nucleotide or amino acid sequence wherein one or more nucleotides or amino acid residues, respectively, have been added.

As used herein, "substitution" refers to a replacement of one or more nucleotides or amino acids by different nucleotides or amino acid residues, respectively.

As used herein, "specifically hybridizable" refers to a nucleic acid or fragment thereof that



hybridizes to another nucleic acid (or a complementary strand thereof) due to the presence of a region that is at least approximately 90% homologous, preferably at least approximately 90-95% homologous, and more preferably approximately 98-100% homologous, as are polynucleotides that hybridize to a partner under stringent hybridization conditions. "Stringent" hybridization conditions are defined  
5 hereinbelow for various hybridization protocols. A probe that is specifically hybridizable to a given sequence can be used to detect a 1 bp out of 10 bp (10%) or a 1 bp out of 20 bp (5%) difference between nucleic acid sequences and is therefore useful for discriminating between a wild type and a mutant form of a gene of interest.

As used herein, "amino acid sequence" refers to the sequential array of amino acids that have  
10 been joined by peptide bonds between the carboxylic acid group of one amino acid and the amino group of the adjacent amino acid to form long linear polymers comprising proteins.

As used herein, "amino acid" refers to protein subunit molecules that contain a carboxylic acid group, and an amino group, both linked to a single carbon atom.

A polypeptide is said to be "encoded" by a polynucleotide if the polynucleotide, either in its  
15 native state or in a recombinant form can be transcribed and/or translated to produce the mRNA for and/or the polypeptide or a fragment thereof.

As used herein, "gene" refers to a region of DNA which includes a portion which can be transcribed into RNA, and which may contain an open reading frame or coding region (also referred to as an exon) which encodes a protein, a non-coding region (also referred to as an intron), and a  
20 specific regulatory region comprising the DNA regulatory elements which control expression of the transcribed region.

As used herein, "coding region" refers to a region of DNA which encodes a protein, also known as an exon.

As used herein, "non-coding region" refers to a region of DNA which does not encode a  
25 protein coding region, also known as an intron, and is not included in the RNA molecule that is synthesized from a particular gene.

As used herein, "regulatory region" refers to DNA sequences which are located either 5' of the transcription start site, 3' or the transcription termination site, within an intron or exon, capable of ensuring that the gene is transcribed at the proper time and in the appropriate cell type.

30 As used herein, "consensus DNA sequence" or "wild-type DNA sequence" refers to a sequence wherein every position represents the nucleotide that occurs with the highest frequency when many actual sequences are compared. As used herein, "consensus DNA sequence" or "wild-type DNA sequence" also refers to the normal, naturally occurring DNA sequence.

As used herein, a given sequence (or mutation or polymorphism) “associated with” osteoarthritis refers to a nucleic acid sequence that increases susceptibility to the disease, predisposes an individual to the disease or contributes to the disease, wherein the nucleic acid sequence is present at a higher frequency (at least 5%, preferably 10%, more preferably 25% higher) in individuals with the disease as compared to individuals who do not have the disease.

As used herein, a sequence “not associated with” osteoarthritis refers to a nucleic acid sequence that does not increase susceptibility to the disease, predispose an individual to the disease or contribute to the disease, wherein the nucleic acid sequence is not present at a higher frequency in individuals with the disease, and thus is present at a frequency about equal to its frequency in individuals who do not have the disease.

As used herein, “amplifying” refers to producing additional copies of a nucleic acid sequence, preferably by the method of polymerase chain reaction (Mullis and Faloona, 1987, Methods Enzymol. 155: 335).

As used herein, “oligonucleotide primers” refer to single stranded DNA or RNA molecules that are hybridizable to a nucleic acid template and prime enzymatic synthesis of a second nucleic acid strand. Oligonucleotide primers useful according to the invention are between 5 to 100 nucleotides in length, preferably 20-60 nucleotides in length, and more preferably 20-40 nucleotides in length.

As used herein, “sequencing” refers to determining the precise nucleotide composition or sequence of a nucleic acid region by methods well known in the art (see Ausubel et al., supra and Sambrook et al., supra).

As used herein, “comparing” a sequence refers to determining if the nucleotides at one or more positions in a particular region of a nucleic acid fragment are identical for any two or more sequences. According to the invention, sequence comparisons can be performed by using computer program analysis as described below in Section F entitled “Identification and Characterization of Polymorphisms”.

As used herein, “sequence differences” or “sequence variations” refer to nucleotide changes, at one or more positions between any two or more sequences being compared.

As used herein, “determining the presence of polymorphic variations” refers to using methods well known in the art to identify a nucleotide, at one or more positions within a particular nucleic acid region, that is distinct from the nucleotide present in the naturally occurring, wild-type or consensus sequence, resulting from either a nucleotide deletion, an insertion or addition, or a substitution.

As used herein, “determining the absence of polymorphic variations” refers to using methods well known in the art to determine that the nucleotides present at every position analyzed in a

particular nucleic acid region are identical to the nucleotides present in the naturally occurring, wild-type or consensus sequence.

As used herein, "genotyping" refers to determining the composition of the genetic material that is inherited by an organism from its parents.

5 As used herein, "biological sample" refers to a tissue or fluid sample containing a polynucleotide or polypeptide of interest, and isolated from an individual including but not limited to plasma, serum, spinal fluid, lymph fluid, urine, stool, external secretions of the skin, respiratory, intestinal and genitorurinary tracts, saliva, blood cells, tumors, organs, tissue and samples of *in vitro* cell culture constituents.

10 As used herein, "amplimers" refer to a specific fragment of DNA generated by PCR that is at least 30 bp in length and is preferably between 50 and 100bp in length, and is more preferably between 150-300bp in length, with a melting temperature in the range of approximately 60-62°C.

As used herein, "phenotype" refers to the biological appearances of an organism or a tissue derived from an organism, wherein biological appearances include chemical, structural and behavioral  
15 attributes, and excludes genetic constitution.

As used herein, "genotype" refers to the genetic material that is inherited by an organism from its parents.

As used herein, "genetic susceptibility to osteoarthritis" refers to an increased risk of developing osteoarthritis resulting from specific DNA differences relative to non-susceptible  
20 individuals. Preferably an individual who is genetically susceptible to osteoarthritis has a 5-100%, and more preferably a 25-50% greater chance of developing osteoarthritis, as compared to non-susceptible individuals.

As used herein, "diagnostic" refers to the practice of identifying a disease from the signs and symptoms of an individual including the DNA sequences of genes that are associated with an  
25 increased susceptibility to the disease. "Diagnostic" also refers to the practice of stratifying patient populations based on the efficacy or toxicity of a composition, and the predictive placement of an individual in a response strata based on stata-associated parameters.

As used herein, "prognosis" refers to the possibility of recovering from a particular disease or condition, and also refers to risk assessment of developing a particular disease or condition.

30

## THE INVENTION

Various embodiments of the invention include polynucleotides and polymorphic polynucleotides associated with a given human disease, for example, with osteoarthritis. The invention also provides a

gene sequence containing one or more polymorphic nucleotides associated with a predisposition to or the development of a given human disease such as osteoarthritis. The invention also relates to polypeptides encoded by the polynucleotides or the polymorphism-containing gene. The invention also provides methods of detecting a polymorphism according to the invention in individuals at risk for  
5 osteoarthritis, and for determining if a given polymorphism is associated with a predisposition to the disease. The invention also discloses polymorphism(s) that are either associated with or are not associated with (i.e., are neutral) osteoarthritis. A polymorphism in a given gene can be utilized in various diagnostic and therapeutic methods and procedures, for example, in nucleic acid and peptide diagnosis, drug screening and design, and in gene and peptide therapy. A polymorphism associated  
10 with a given gene can be utilized in various gene expression systems and assays designed to analyze gene regulation and expression.

#### A. Design and Synthesis of Oligonucleotide Primers

According to the present invention, oligonucleotide primers are disclosed that are useful for  
15 determining the sequence of a particular allele of a gene. The invention also discloses oligonucleotide primers designed to amplify a region of a gene that is known to contain a polymorphism. The invention also discloses oligonucleotide primers designed to anneal specifically to a particular allele of a gene.

Oligonucleotide primers useful according to the invention are single-stranded DNA or RNA  
molecules that are hybridizable to a nucleic acid template and prime enzymatic synthesis of a second  
20 nucleic acid strand. The primer is complementary to a portion of a target molecule present in a pool of nucleic acid molecules. It is contemplated that oligonucleotide primers according to the invention are prepared by synthetic methods, either chemical or enzymatic. Alternatively, such a molecule or a fragment thereof is naturally-occurring, and is isolated from its natural source or purchased from a commercial supplier. Oligonucleotide primers are 5 to 100 nucleotides in length, ideally from 20 to 40  
25 nucleotides, although oligonucleotides of different length are of use.

Pairs of single-stranded DNA primers can be annealed to sequences within or surrounding a gene on chromosome Y in order to prime amplifying DNA synthesis of a region of a gene. A complete set of gene primers will allow synthesis of all of the nucleotides of the coding sequences, e.g., the exons, introns and control regions. Preferably, the set of primers will also allow synthesis of  
30 both intron and exon sequences.

Allele-specific primers are also useful, according to the invention. Such primers will anneal only to a particular-mutant allele (e.g. alleles containing a polymorphism), and thus will only amplify a product if the template also contains the polymorphism. Allele specific primers that anneal only to a

wild type gene sequence are also useful according to the invention.

Typically, selective hybridization occurs when two nucleic acid sequences are substantially complementary (at least about 65% complementary over a stretch of at least 14 to 25 nucleotides, preferably at least about 75%, more preferably at least about 90% complementary). See Kanehisa, M., 1984, Nucleic Acids Res. 12: 203, incorporated herein by reference. As a result, it is expected that a certain degree of mismatch at the priming site is tolerated. Such mismatch may be small, such as a mono-, di- or tri-nucleotide. Alternatively, it may encompass loops, which are defined as regions in which there exists a mismatch in an uninterrupted series of four or more nucleotides.

Numerous factors influence the efficiency and selectivity of hybridization of the primer to a second nucleic acid molecule. These factors, which include primer length, nucleotide sequence and/or composition, hybridization temperature, buffer composition and potential for steric hindrance in the region to which the primer is required to hybridize, will be considered when designing oligonucleotide primers according to the invention.

A positive correlation exists between primer length and both the efficiency and accuracy with which a primer will anneal to a target sequence. In particular, longer sequences have a higher melting temperature ( $T_M$ ) than do shorter ones, and are less likely to be repeated within a given target sequence, thereby minimizing promiscuous hybridization. Primer sequences with a high G-C content or that comprise palindromic sequences tend to self-hybridize, as do their intended target sites, since unimolecular, rather than bimolecular, hybridization kinetics are generally favored in solution.

However, it is also important to design a primer that contains sufficient numbers of G-C nucleotide pairings since each G-C pair is bound by three hydrogen bonds, rather than the two that are found when A and T bases pair to bind the target sequence, and therefore forms a tighter, stronger bond. Hybridization temperature varies inversely with primer annealing efficiency, as does the concentration of organic solvents, e.g. formamide, that might be included in a priming reaction or hybridization mixture, while increases in salt concentration facilitate binding. Under stringent annealing conditions, longer hybridization probes (of use, for example, in Northern analysis), or synthesis primers, hybridize more efficiently than do shorter ones, which are sufficient under more permissive conditions. Stringent hybridization conditions typically include salt concentrations of less than about 1M, more usually less than about 500 mM and preferably less than about 200 mM. Hybridization temperatures range from as low as 0°C to greater than 22°C, greater than about 30°C, and (most often) in excess of about 37°C. Longer fragments may require higher hybridization temperatures for specific hybridization. As several factors affect the stringency of hybridization, the combination of parameters is more important than the absolute measure of a single factor.

Oligonucleotide primers can be designed with these considerations in mind and synthesized according to the following methods.

#### 1. Oligonucleotide Primer Design Strategy

5 The design of a particular oligonucleotide primer for the purpose of sequencing or PCR involves selecting a sequence that is capable of recognizing the target sequence, but has a minimal predicted secondary structure. The oligonucleotide sequence binds only to a single site in the target nucleic acid. Furthermore, the  $T_m$  of the oligonucleotide is optimized by analysis of the length and GC content of the oligonucleotide. Furthermore, when designing a PCR primer useful for the amplification  
10 of genomic DNA, the selected primer sequence does not demonstrate significant matches to sequences in the GenBank database (or other available databases).

The design of a primer is facilitated by the use of readily available computer programs, developed to assist in the evaluation of the several parameters described above and the optimization of primer sequences. Examples of such programs are "PrimerSelect" of the DNASTar™ software  
15 package (DNASTar, Inc.; Madison, WI), OLIGO 4.0 (National Biosciences, Inc.), PRIMER, Oligonucleotide Selection Program, PGEN and Amplify (described in Ausubel et al., 1995, Short Protocols in Molecular Biology, 3rd Edition, John Wiley & Sons). Primers are designed with sequences that serve as targets for other primers to produce a PCR product that has known  
sequences on the ends which serve as targets for further amplification (e.g. to sequence the PCR  
20 product). If many different genes are amplified with specific primers that share a common 'tail' sequence', the PCR products from these distinct genes can subsequently be sequenced with a single set of primers. Alternatively, in order to facilitate subsequent cloning of amplified sequences, primers are designed with restriction enzyme site sequences appended to their 5' ends. Thus, all nucleotides of the primers are derived from gene sequences or sequences adjacent to a gene, except for the few  
25 nucleotides necessary to form a restriction enzyme site. Such enzymes and sites are well known in the art. If the genomic sequence of a gene and the sequence of the open reading frame of a gene are known, design of particular primers is well within the skill of the art.

#### 2. Synthesis

The primers themselves are synthesized using techniques which are also well known in the  
30 art. Once designed, oligonucleotides are prepared by a suitable method, e.g. the phosphoramidite method described by Beaucage and Carruthers (1981, Tetrahedron Lett., 22:1859) or the triester method according to Matteucci et al. (1981, J. Am. Chem. Soc., 103:3185), both incorporated herein by reference, or by other chemical methods using either a commercial automated oligonucleotide

synthesizer (which is commercially available) or VLSIPS™ technology.

## B. Production of a Polynucleotide Sequence

The invention discloses polynucleotide sequences comprising polymorphisms. The polynucleotide sequences of the invention are specifically hybridizable to a mutant form of a gene and are therefore useful for discriminating between a wild-type form of a gene and a mutant form of a gene. The polynucleotide sequences of the invention may also be useful for expression of the encoded protein or a fragment thereof. The invention also features antisense polynucleotide sequences complementary to polynucleotide sequences comprising polymorphisms. Antisense polynucleotide sequences are useful according to the invention for inhibiting expression of an allelic form of a gene.

The present invention utilizes polynucleotide sequences and fragments comprising RNA, cDNA, genomic DNA, synthetic forms, and mixed polymers. The invention includes both sense and antisense strands of the polynucleotide sequences. According to the invention, the polynucleotide sequences may be chemically or biochemically modified or may contain non-natural or derivatized nucleotide bases. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages (e.g. methyl phosphonates, phosphorodithioates, etc.), pendent moieties (e.g., polypeptides), intercalators, (e.g. acridine, psoralen, etc.) chelators, alkylators, and modified linkages (e.g. alpha anomeric nucleic acids, etc.) Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule.

The polynucleotide may be a naturally occurring polynucleotide, or may be a structurally related variant of such a polynucleotide having modified bases and/or sugars and/or linkages. The term "polynucleotide" as used herein is intended to cover all such variants.

Modifications, which may be made to the polynucleotide may include (but are not limited to) the following types:

### a) Backbone modifications

i) phosphorothioates (X or Y or W or Z = S or any combination of two or more with the remainder as O).

e.g. Y=S (Stein et al., 1988, Nucleic Acids Res., 15:3209), X=S (Cosstick and Vyle, 1989, Tetrahedron Letters, 30:4693), Y and Z=S (Brill et al., 1989, J. Amer. Chem. Soc., 111:2321)

ii) methylphosphonates (eg Z=methyl (Miller et al., 1980, J. Biol. Chem., 255:9569))

iii) phosphoramidates ( $Z = N-(\text{alkyl})_2$  e.g. alkyl methyl, ethyl, butyl) ( $Z = \text{morpholine}$  or piperazine) (Agrawal et al., 1988, Proc. Natl. Acad. Sci. USA, 85:7079) ( $X$  or  $W = NH$ ) (Mag and Engels, 1988, Nucleic Acids Res., 16:3525)

iv) phosphotriesters ( $Z = O\text{-alkyl}$  e.g. methyl, ethyl etc) (Miller et al., Biochemistry, 21:5468)

v) phosphorus-free linkages (e.g. carbamate, acetamidate, acetate) (Gait et al., 1974, J Chem.Soc. Perkin I, 1684, Gait et al., 1979, J Chem.Soc. Perkin I, 1389)

b) Sugar modifications

i) 2'-deoxynucleosides ( $R = H$ )

ii) 2'-O-methylated nucleosides ( $R = OMe$ ) (Sproat et al., 1989, Nucleic Acids Res., 17:

3373)

iii) 2'-fluoro-2'-deoxynucleosides ( $R = F$ ) (Krug et al., 1989, Nucleosides and Nucleotides, 8:1473)

c) Base modifications - (for a review see Jones, 1979, Int. J. Biolog. Macromolecules, 1:194)

i) pyrimidine derivatives substituted in the 5-position (e.g. methyl, bromo, fluoro etc) or replacing a carbonyl group by an amino group (Piccirilli et al., 1990, Nature, 343:33).

ii) purine derivatives lacking specific nitrogen atoms (e.g. 7-deaza adenine, hypoxanthine) or functionalized in the 8-position (e.g. 8-azido adenine, 8-bromo adenine)

d) Polynucleotides covalently linked to reactive functional groups, e.g.:

i) psoralens (Miller et al., 1988, Nucleic Acids Res. Special Pub. No. 20:113, phenanthrolines

(Sun et al., 1988, Biochemistry, 27:6039), mustards (Vlassov et al., 1988, Gene, 72:313) (irreversible cross-linking agents with or without the need for co-reagents)

ii) acridine (intercalating agents) (Helene et al., 1985, Biochimie, 67:777)

iii) thiol derivatives (reversible disulphide formation with proteins) (Connolly and Newman, 1989, Nucleic Acids Res., 17:4957)

iv) aldehydes (Schiffs base formation)

v) azido, bromo groups (UV cross-linking)

vi) ellipticines (photolytic cross-linking) (Perrouault et al., 1990, Nature, 344:358)

e) Polynucleotides covalently linked to lipophilic groups or other reagents capable of improving uptake by cells, e.g.:

i) cholesterol (Letsinger et al., 1989, Proc. Natl. Acad. Sci. USA, 86:6553), polyamines (Lemaitre et al., 1987, Proc. Natl. Acad. Sci. USA, 84: 648), other soluble polymers (e.g. polyethylene glycol)

f) Polynucleotides containing alpha-nucleosides (Morvan et al., Nucleic Acids Res., 15: 3421)



g) Combinations of modifications a)-f)

It should be noted that such modified polynucleotides, while sharing features with polynucleotides designed as “anti-sense” inhibitors, are distinct in that the compounds correspond to sense-strand sequences and the mechanism of action depends on protein-nucleic acid interactions and does not depend upon interactions with nucleic acid sequences.

1. Polynucleotide Sequences Comprising DNA

a. Cloning

Polynucleotide sequences comprising DNA can be isolated from cDNA or genomic libraries (including YAC and BAC libraries) by cloning methods well known to those skilled in the art (Ausubel et al., supra). Briefly, isolation of a DNA clone comprising a particular polynucleotide sequence involves screening a recombinant DNA or cDNA library and identifying the clone containing the desired sequence. Cloning will involve the following steps. The clones of a particular library are spread onto plates, transferred to an appropriate substrate for screening, denatured, and probed for the presence of a particular sequence. A description of hybridization conditions, and methods for producing labeled probes is included below.

The desired clone is preferably identified by hybridization to a nucleic acid probe or by expression of a protein that can be detected by an antibody. Alternatively, the desired clone is identified by polymerase chain amplification of a sequence defined by a particular set of primers according to the methods described below.

The selection of an appropriate library involves identifying tissues or cell lines that are an abundant source of the desired sequence. Furthermore, if the polynucleotide sequence of interest contains regulatory sequence or intronic sequence a genomic library is screened (Ausubel et al., supra).

b. Genomic DNA

Polynucleotide sequences of the invention are amplified from genomic DNA. Genomic DNA is isolated from tissues or cells according to the following method.

To facilitate detection of a variant form of a gene from a particular tissue, the tissue is isolated free from surrounding normal tissues. To isolate genomic DNA from mammalian tissue, the tissue is minced and frozen in liquid nitrogen. Frozen tissue is ground into a fine powder with a prechilled mortar and pestle, and suspended in digestion buffer (100 mM NaCl, 10 mM TrisCl, pH 8.0, 25 mM EDTA, pH 8.0, 0.5% (w/v) SDS, 0.1 mg/ml proteinase K) at 1.2ml digestion buffer per 100mg of tissue. To isolate genomic DNA from mammalian tissue culture cells, cells are pelleted by

centrifugation for 5 min at 500 x g, resuspended in 1-10 ml ice-cold PBS, repelleted for 5 min at 500 x g and resuspended in 1 volume of digestion buffer.

Samples in digestion buffer are incubated (with shaking) for 12-18 hours at 50°C, and then extracted with an equal volume of phenol/chloroform/isoamyl alcohol. If the phases are not resolved following a centrifugation step (10 min at 1700 x g), another volume of digestion buffer (without proteinase K) is added and the centrifugation step is repeated. If a thick white material is evident at the interface of the two phases, the organic extraction step is repeated. Following extraction the upper, aqueous layer is transferred to a new tube to which will be added 1/2 volume of 7.5M ammonium acetate and 2 volumes of 100% ethanol. The nucleic acid is pelleted by centrifugation for 2 min at 1700 x g, washed with 70% ethanol, air dried and resuspended in TE buffer (10 mM TrisCl, pH 8.0, 1 mM EDTA, pH 8.0) at 1mg/ml. Residual RNA is removed by incubating the sample for 1 hour at 37°C in the presence of 0.1% SDS and 1 mg/ml DNase-free RNASE, and repeating the extraction and ethanol precipitation steps. The yield of genomic DNA, according to this method is expected to be approximately 2 mg DNA/1 g cells or tissue (Ausubel et al., supra). Genomic DNA isolated according to this method can be used for Southern blot analysis, restriction enzyme digestion, dot blot analysis or PCR analysis, according to the invention.

c. Restriction digest (of cDNA or genomic DNA)

Following the identification of a desired cDNA or genomic clone containing a particular sequence, polynucleotides of the invention are isolated from these clones by digestion with restriction enzymes.

The technique of restriction enzyme digestion is well known to those skilled in the art (Ausubel et al., supra). Reagents useful for restriction enzyme digestion are readily available from commercial vendors including New England Biolabs, Boehringer Mannheim, Promega, as well as other sources.

d. PCR

Polynucleotide sequences of the invention are amplified from genomic DNA or other natural sources by the polymerase chain reaction (PCR). PCR methods are well-known to those skilled in the art.

PCR provides a method for rapidly amplifying a particular DNA sequence by using multiple cycles of DNA replication catalyzed by a thermostable, DNA-dependent DNA polymerase to amplify the target sequence of interest. PCR requires the presence of a nucleic acid to be amplified, two single stranded oligonucleotide primers flanking the sequence to be amplified, a DNA polymerase, deoxyribonucleoside triphosphates, a buffer and salts.

The method of PCR is well known in the art. PCR, is performed as described in Mullis and

Faloona, 1987, Methods Enzymol., 155: 335, herein incorporated by reference.

PCR is performed using template DNA (at least 1 fg; more usefully, 1 - 1000 ng) and at least 25 pmol of oligonucleotide primers. A typical reaction mixture includes: 2 ml of DNA, 25 pmol of oligonucleotide primer, 2.5 ml of IOx PCR buffer 1 (Perkin-Elmer, Foster City, CA), 0.4 ml of 1.25 mM dNTP, 0.15 ml (or 2.5 units) of Taq DNA polymerase (Perkin Elmer, Foster City, CA) and deionized water to a total volume of 25 ml. Mineral oil is overlaid and the PCR is performed using a programmable thermal cycler.

The length and temperature of each step of a PCR cycle, as well as the number of cycles, are adjusted according to the stringency requirements in effect. Annealing temperature and timing are determined both by the efficiency with which a primer is expected to anneal to a template and the degree of mismatch that is to be tolerated. The ability to optimize the stringency of primer annealing conditions is well within the knowledge of one of moderate skill in the art. An annealing temperature of between 30°C and 72°C is used. Initial denaturation of the template molecules normally occurs at between 92°C and 99°C for 4 minutes, followed by 20-40 cycles consisting of denaturation (94-99°C for 15 seconds to 1 minute), annealing (temperature determined as discussed above; 1-2 minutes), and extension (72°C for 1 minute). The final extension step is generally carried out for 4 minutes at 72°C, and may be followed by an indefinite (0-24 hour) step at 4°C.

Several techniques for detecting PCR products quantitatively without electrophoresis may be useful according to the invention in order to make it more suitable for easy clinical use. One of these techniques, for which there are commercially available kits such as Taqman™ (Perkin Elmer, Foster City, CA), is performed with a transcript-specific antisense probe. This probe is specific for the PCR product (e.g. a nucleic acid fragment derived from a gene) and is prepared with a quencher and fluorescent reporter probe complexed to the 5' end of the oligonucleotide. Different fluorescent markers can be attached to different reporters, allowing for measurement of two products in one reaction. When Taq DNA polymerase is activated, it cleaves off the fluorescent reporters of the probe bound to the template by virtue of its 5'-to-3' nucleolytic activity. In the absence of the quenchers, the reporters now fluoresce. The color change in the reporters is proportional to the amount of each specific product and is measured by a fluorometer; therefore, the amount of each color can be measured and the PCR product can be quantified. The PCR reactions can be performed in 96 well plates so that samples derived from many individuals can be processed and measured simultaneously. The Taqman™ system has the additional advantage of not requiring gel electrophoresis and allows for quantification when used with a standard curve.

## 2. Polynucleotide Sequences Comprising RNA

The present invention also provides a polynucleotide sequence comprising RNA. A polynucleotide comprising RNA is useful for detecting snps and polymorphisms by techniques including but not limited to hybridization methods or the RNase protection method. A polynucleotide comprising RNA is also useful as a template for the *in vitro* production of protein. A polynucleotide comprising RNA is also useful for detecting and localizing specific mRNA sequences by *in situ* hybridization.

Polynucleotide sequences comprising RNA can be produced according to the method of *in vitro* transcription.

The technique of *in vitro* transcription is well known to those of skill in the art. Briefly, the gene of interest is inserted into a vector containing an SP6, T3 or T7 promoter. The vector is linearized with an appropriate restriction enzyme that digests the vector at a single site located downstream of the coding sequence. Following a phenol/chloroform extraction, the DNA is ethanol precipitated, washed in 70% ethanol, dried and resuspended in sterile water. The *in vitro* transcription reaction is performed by incubating the linearized DNA with transcription buffer (200 mM TrisCl, pH 8.0, 40 mM MgCl<sub>2</sub>, 10 mM spermidine, 250 NaCl [T7 or T3] or 200 mM TrisCl, pH 7.5, 30 mM MgCl<sub>2</sub>, 10 mM spermidine [SP6]), dithiothreitol, RNase inhibitors, each of the four ribonucleoside triphosphates, and either SP6, T7 or T3 RNA polymerase for 30 min at 37°C. To prepare a radiolabeled polynucleotide comprising RNA, unlabeled UTP will be omitted and  $\alpha$ -SUTP will be included in the reaction mixture. The DNA template is then removed by incubation with DNaseI. Following ethanol precipitation, an aliquot of the radiolabeled RNA is counted in a scintillation counter to determine the cpm/ml (Ausubel et al., *supra*).

Alternatively, polynucleotide sequences comprising RNA are prepared by chemical synthesis techniques such as solid phase phosphoramidite (described above).

### 3. Polynucleotide Sequences Comprising Oligonucleotides

A polynucleotide sequence comprising oligonucleotides can be made by using oligonucleotide synthesizing machines which are commercially available (described above).

### 4. Polynucleotide Sequences Encoding Fusion Proteins

Polynucleotide sequences of the invention can be used to express the protein product (or fragment thereof) of the gene of interest by inserting the polynucleotide sequence into an expression vector. Expression vectors suitable for protein expression in mammalian cells, bacterial cells, insect cells or plant cells are well known in the art and are described in Section H entitled "Production of a Mutant Protein".

Polynucleotide sequences of the invention can be used to prepare hybrid polynucleotides

comprising a sequence of a gene adjacent to a sequence encoding a foreign protein or a fragment thereof (e.g. lacZ, trpE, glutathione S-transferase or thioredoxin) or a protein tag (hemmagglutinin or FLAG). Such hybrid polynucleotides produce fusion proteins that are useful, according to the invention, for improved expression and/or rapid isolation of a protein or protein fragment, encoded by the sequence of a gene. Hybrid polynucleotides are also useful as a source of antigen for the production of antibodies.

Nucleic acid constructs comprising a polynucleotide of genomic, cDNA, synthetic or semi-synthetic origin in association with a polynucleotide sequence encoding a foreign protein or a fragment thereof, (carrier sequence) can be generated by recombinant nucleic acid techniques well known in the art (See Ausubel et al., supra). According to this method, the cloned gene is introduced into an expression vector at a position located 3' to a carrier sequence coding for the amino terminus of a highly expressed protein, an entire functional moiety of a highly expressed protein or the entire protein. It is preferable to use a carrier sequence from an *E. coli* gene or from any gene that is expressed at high levels in *E. coli*. It is often preferable to select a carrier sequence that will facilitate protein purification, either with antibodies, or with an affinity purification protocol that is specific for the carrier protein being used. For example, the purification protocol can be designed in accordance with the unique physical properties of the carrier protein (e.g. heat stability). Alternatively, the tag sequence may encode a protein (e.g. glutathione-S-transferase (GST)) which can be purified by either a chemical interaction (for example glutathione purification of GST). Alternatively, some carrier proteins, such as thioredoxin (Trx) can be selectively released from intact cells by osmotic shock or freeze/thaw procedures. Often, proteins that are fused to these carrier proteins can be purified away from intracellular contaminants by virtue of the physical attributes of the carrier protein (Ausubel et al., supra).

To ensure that a fusion protein is useful, according to the invention, it may be necessary to modify the expression protocol to produce a soluble protein. Due to the fact that high-level expression of certain proteins can lead to the formation of inclusion bodies, if a soluble protein is required it may be necessary to modify the following variables. The temperature at which expression is induced can affect inclusion body formation since inclusion body formation is induced at higher temperatures (37°C and 42°C) and inhibited at lower temperatures (30°C). In certain instances, lowering the total level of protein expression can lead to an increase in the proportion of soluble protein that is produced. The strain background of the cells in which the protein is being produced can affect the proportion of a particular protein that is expressed in a soluble form. Furthermore, the choice of carrier protein can affect the solubility of an expressed fusion protein (Ausubel et al., supra).

An additional problem that can be encountered when producing fusion proteins in *E. coli* is formation of an unstable protein, or a protein that is cleaved at the site of the junction between the carrier sequence and the sequence of the protein of interest. To decrease complications due to protein instability one can arrange for the fusion protein to be expressed as insoluble aggregates. Alternatively, one can express the fusion protein in *E. coli* strains that are deficient in proteases (Ausubel et al., supra).

Often it is useful to remove the carrier protein moiety from the protein of interest to facilitate biochemical and functional analyses. Methods for cleavage of fusion proteins to remove the carrier are known to those skilled in the art. The choice of a method is usually determined by the composition, sequence, and physical characteristics of the particular protein. Reagents such as cyanogen bromide, hydroxylamine or low pH can be used to chemically cleave fusion proteins. To avoid complications resulting from chemical cleavage (e.g. the presence of chemical cleavage sites in the protein of interest and/or the occurrence of side reactions resulting in protein modification), enzymatic cleavage methods can be used. Enzymatic cleavage protocols are advantageous because they can be carried out under relatively mild reaction conditions, and because they involve highly specific cleavage reactions. Enzymes useful for enzymatic cleavage of fusion proteins include factor Xa, thrombin, enterokinase, renin and collagenase (Ausubel et al., supra).

Recombinant constructs encoding fusion proteins wherein the carrier sequence is on the order of 9-15 codons, can be generated by PCR methods. According to this method, a PCR primer will be designed to contain at least 13 nucleotides that are identical to the target sequence on either side of the nucleotide sequence encoding the carrier sequence. Preferably, the PCR primer will also contain a restriction enzyme site to facilitate cloning of the amplified product into an appropriate expression vector. PCR will be carried out as described above and the sequence of the amplified product will be confirmed by sequence analysis as described in Section D entitled "Isolation of a Wild type Gene".

Alternatively, recombinant constructs encoding fusion proteins can be generated by site/oligonucleotide directed mutagenesis (Ausubel et al., supra). According to the method of site directed mutagenesis the DNA to be mutated is inserted into a plasmid which has an F1 origin of replication. A mutagenesis oligonucleotide is designed to contain 13 bp that are 100% identical to the target sequence, on either side of a sequence coding for the 9-15 codons of carrier sequence that is to be added by the mutagenesis protocol.

A single stranded preparation of the vector is prepared by the following method. Following transformation of an appropriate bacterial strain (e.g. CJ236) with the recombinant plasmid and plating of the bacteria on LB agar plates, a single resulting colony is grown in 4x5 ml of LB plus ampicillin for

1 hour at 37°C with vigorous shaking. M13K07 helper phage (2 ml, approximately  $10^{10}$ - $10^{11}$  plaque forming units) is added and the bacteria are grown for an additional hour at 37°C with vigorous shaking. Following the addition of 7 ml of kanamycin (50 mg/ml), the bacteria are grown overnight at 37°C with vigorous shaking. The following day bacterial cultures are pooled and cells are separated by centrifugation. After the addition of 2.6 ml of 20% polyethylene glycol 200-800/2M NaCl to 20 ml of bacterial supernatant, the sample is incubated for 1 - 1.5 hours on ice. The sample is pelleted by centrifugation at 9000 rpm for 20 minutes. Following removal of the supernatant, residual supernatant are removed by centrifugation at 3000 rpm for 5 minutes. The pellet is resuspended in 400 ml of TE, extracted twice with phenol and four times with phenol:chloroform and ethanol precipitated. The resulting pellet is resuspended in 40 ml TE.

Mutagenesis is performed by using a muta-gene kit (Bio-Rad, Hercules, CA) according to the following method. To kinase the oligonucleotide primer, 1 ml (200ng) of oligonucleotide is incubated in the presence of 2 ml of 10 kinase buffer (0.5M Tris, pH 8.0, 70mM  $MgCl_2$ , 10mM DTT), 2 ml 10mM rATP, 2 ml polynucleotide kinase and 13 ml  $H_2O$  for 37°C for 1 hour. To carry out the annealing and synthesis steps, 2.5 ml of single-stranded template are mixed with 1 ml of kinased oligonucleotide, 1.0 ml of 10X annealing buffer (200mM Tris-HCl, pH 7.4, 20 mM  $MgCl_2$ , 500mM NaCl) and 5.5 ml  $H_2O$  for 10 min at 65°C. The reaction mixture is slow-cooled to 37°C. Once the sample has reached 37°C, the sample is spun briefly in a microfuge. Following the addition of 1.0 ml of 10X synthesis buffer (5mM each dATP, dCTP, cGTP, dTTP, 10mM ATP, 100mM Tris-HCl, pH 7.4, 50 mM  $MgCl_2$ , 20mM DTT), 1.0 ml T4 DNA ligase and 0.5 ml of T4 DNA polymerase, the sample is incubated for 5 minutes on ice, 5 minutes at room temperature and 1 hour at 37°C. A 2 ml aliquot of the sample is used to transform *E. coli*.

DNA is isolated from the transformed *E. coli* cells by mini prep methods known in the art (Ausubel et al., supra), and sequenced according to methods known in the art (described in Section D entitled "Isolation of a Wild Type Gene").

### C. Production of a Nucleic acid Probe

The invention discloses nucleic acid probes. Preferably, the nucleic acid probes of the invention are specifically hybridizable to a mutant gene but not to a wild type form of a gene due to the presence of one or more polymorphisms. These allele specific probes can be used to screen DNA sequences of a gene which have been amplified by PCR, or are present in a genomic DNA or RNA test sample. Hybridization of a particular allele specific probe to an amplified gene sequence, under stringent conditions (described below), indicates that the polymorphism contained in the probe is

present in the amplified sequence. Hybridization of a particular allele specific probe to a test sample comprising genomic DNA or RNA, under stringent conditions (described below), indicates that the polymorphism contained in the probe, is present in the nucleic acid of the test sample. Nucleic acid probes that are specifically hybridizable to a wild type form of a gene but not to a mutant form of a gene are also useful according to the invention.

In another embodiment, the probes of the claimed invention will be specific for a nucleic acid region that is adjacent to a region that is thought to contain one or more polymorphisms. These probes will be useful for detecting the presence of one or more polymorphisms in the adjacent region by the method of primer extension (as described in Section F entitled "Identification and Characterization of Polymorphisms").

In other embodiments, probes of the claimed invention will be used to detect a gain or loss of a restriction enzyme site known to contain one or more polymorphisms of the claimed invention. Nucleic acid probes, according to this embodiment, are able to detect a restriction enzyme fragment that is of a size that can be easily separated on an agarose gel and visualized by Southern blot analysis. Probes that are useful according to this embodiment of the claimed invention can be specific for any region within a gene or outside of a gene.

The nucleic acids probes of the invention are useful for a variety of hybridization-based analyses including but not limited to Southern hybridization to genomic DNA, cDNA sequences or PCR amplification products; Northern hybridization to mRNA and RNase protection assays, DNA sequencing and isolation of genomic or cDNA clones of a gene. The probes may also be used to determine whether mRNA encoded for by a gene is present in a cell or tissue by the method of *in situ* hybridization. These techniques are well known in the art and can be performed as described in Ausubel et al., *supra*.

According to the methods of the above-referenced hybridization assays, polymorphisms associated with alleles of a gene, which either predispose to a particular disease (e.g. osteoarthritis) or are not associated with a particular disease (e.g. osteoarthritis), will be detected by the formation of a stable hybrid consisting of a polynucleotide probe comprising one or more polymorphisms and a target sequence, that also comprises one or more polymorphisms, under stringent to moderately stringent hybridization and wash conditions. If it is expected that the probes will be perfectly complementary to the target sequence, stringent conditions will be used. Hybridization stringency may be lessened if some mismatching is expected, for example, if variants are expected with the result that the probe will not be completely complementary. Conditions are chosen which rule out nonspecific/adventitious bindings, that is, which minimize noise. Since such indications identify neutral DNA polymorphisms as



well as mutations, these indications need further analysis (such as assays described in Section F entitled "Identification and Characterization of Polymorphisms") to demonstrate detection of a susceptibility allele of a gene.

Probes for alleles of a gene may be derived from genomic DNA or cDNA sequences from  
5 specific for the gene of interest. The probes may be of any suitable length, which span all or a portion of the region containing the gene. If the target sequence contains a sequence identical to that of the probe, the probes may be short, e.g., in the range of about 8-30 base pairs, since the hybrid will be relatively stable under even stringent conditions. If some degree of mismatch is expected with the probe, i.e., if it is suspected that the probe will hybridize to a variant region, a longer probe may be  
10 employed which hybridizes to the target sequence with the requisite specificity.

Probes according to the invention also include an isolated polynucleotide attached to a label or a reporter molecule which may be useful for isolating other polynucleotide sequences, having sequence similarity by standard methods, including but not limited to the above-referenced hybridization-based assays. Techniques for preparing and labeling probes (as described in Ausubel et  
15 al. Supra) are included below. A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in a various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting related sequences include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the protein-encoding sequence, or any portion of it, may be cloned into a vector for the production of an mRNA  
20 probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labeled nucleotides.

A number of companies such as Pharmacia Biotech (Piscataway NJ), Promega (Madison WI) and US Biochemical Corp (Cleveland OH) supply commercial kits and protocols for these  
25 procedures. Suitable reporter molecules or labels include those radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles and the like. Patents teaching the use of such labels include US Patents 3,817,838; 3,350,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241. Also, recombinant immunoglobulins may be produced as shown in US Patent No. 4,816,567 incorporated herein by reference.

30 Probes comprising synthetic oligonucleotides or other polynucleotides of the present invention may be derived from naturally occurring or recombinant single- or double- stranded polynucleotides, or be chemically synthesized.

Portions of the polynucleotide sequence having at least approximately 5 nucleotides,

preferably 9-15 nucleotides, fewer than about 6 kb and usually fewer than about 1 kb, from a polynucleotide sequence encoding a gene are preferred as probes.

A DNA probe useful according to the present invention can be isolated from a gene or a polynucleotide construct derived from a gene, or from a cDNA sequence specific for a gene or a cDNA construct specific for a gene by the methods of PCR or restriction enzyme digestion, as described above. Riboprobes useful according to the invention can be synthesized by the method of *in vitro* transcription, or by chemical synthesis methods, as described above.

An oligonucleotide probe useful according to the invention can be designed, as described above, and synthesized in a commercially available automated synthesizer.

Nucleic acid hybridization rate and stability will be affected by a variety of experimental parameters including salt concentration, temperature, the presence of organic solvents, the viscosity of the hybridization solution, the base composition of the probe, the length of the duplex, and the number of mismatches between the hybridizing nucleic acids (Ausubel et al., *supra*), and as described in Section A entitled "Design and Synthesis of Oligonucleotide Primers".

Southern blot analysis can be used to detect sequence variations in a gene from a PCR amplified product or from a total genomic DNA test sample via a non-PCR based assay. The method of Southern blot analysis is well known in the art (Ausubel et al., *supra*, Sambrook et al., 1989, Molecular Cloning. A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). This technique involves the transfer of DNA fragments from an electrophoresis gel to a membrane support resulting in the immobilization of the DNA fragments. The resulting membrane carries a semipermanent reproduction of the banding pattern of the gel.

Southern blot analysis is performed according to the following method. Genomic DNA (5-20 mg) is digested with the appropriate restriction enzyme and separated on a 0.6-1.0% agarose gel in TAE buffer. The DNA is transferred to a commercially available nylon or nitrocellulose membrane (e.g. Hybond-N membrane, Amersham, Arlington Heights, IL) by methods well known in the art (Ausubel et al., *supra*, Sambrook et al., *supra*). Following transfer and UV cross linking, the membrane is hybridized with a radiolabeled probe in hybridization solution (e.g. under stringent conditions in 5X SSC, 5X Denhardt solution, 1% SDS) at 65°C. Alternatively, high stringency hybridization can be performed at 68°C or in a hybridization buffer containing a decreased concentration of salt, for example 0.1X SSC. The hybridization conditions can be varied as necessary according to the parameters described in Section A entitled "Design and Synthesis of Oligonucleotide Primers". Following hybridization, the membrane is washed at room temperature in 2X SSC/0.1% SDS and at 65°C in 0.2X SSC/0.1% SDS, and exposed to film. The stringency of the wash buffers can also be

varied depending on the amount of the background signal (Ausubel et al., supra).

Detection of a nucleic acid probe-target nucleic acid hybrid will include the step of hybridizing a nucleic acid probe to the DNA target. This probe may be radioactively labeled or covalently linked to an enzyme such that the covalent linkage does not interfere with the specificity of the hybridization.

5 A resulting hybrid can be detected with a labeled probe. Methods for radioactively labeling a probe include random oligonucleotide primed synthesis, nick translation or kinase reactions (see Ausubel et al., supra). Alternatively, a hybrid can be detected via non-isotopic methods. Non-isotopically labeled probes can be produced by the addition of biotin or digoxigenin, fluorescent groups, chemiluminescent groups (e.g. dioxetanes, particularly triggered dioxetanes), enzymes or antibodies. Typically, non-  
10 isotopic probes are detected by fluorescence or enzymatic methods. Detection of a radiolabeled probe-target nucleic acid complex can be accomplished by separating the complex from free probe and measuring the level of complex by autoradiography or scintillation counting. If the probe is covalently linked to an enzyme, the enzyme-probe-conjugate- target nucleic acid complex will be isolated away from the free probe enzyme conjugate and a substrate will be added for enzyme  
15 detection. Enzymatic activity will be observed as a change in color development or luminescent output resulting in a  $10^3$ - $10^6$  increase in sensitivity. An example of the preparation and use of nucleic acid probe-enzyme conjugates as hybridization probes (wherein the enzyme is alkaline phosphatase) is described in (Jablonski et al., 1986, Nucleic Acids Res., 14:6115)

Two-step label amplification methodologies are known in the art. These assays are based on  
20 the principle that a small ligand (such as digoxigenin, biotin, or the like) is attached to a nucleic acid probe capable of specifically binding to a gene. Allele specific gene probes are also useful according to this method.

According to the method of two-step label amplification, the small ligand attached to the nucleic acid probe will be specifically recognized by an antibody-enzyme conjugate. For example,  
25 digoxigenin will be attached to the nucleic acid probe and hybridization will be detected by an antibody-alkaline phosphatase conjugate wherein the alkaline phosphatase reacts with a chemiluminescent substrate. For methods of preparing nucleic acid probe-small ligand conjugates, see (Martin et al., 1990, BioTechniques, 9:762). Alternatively, the small ligand will be recognized by a second ligand-enzyme conjugate that is capable of specifically complexing to the first ligand. A well known example  
30 of this manner of small ligand interaction is the biotin avidin interaction. Methods for labeling nucleic acid probes and their use in biotin-avidin based assays are described in Rigby et al., 1977, J. Mol. Biol., 113:237 and Nguyen et al., 1992, BioTechniques, 13:116).

Variations of the basic hybrid detection protocol are known in the art, and include

modifications that facilitate separation of the hybrids to be detected from extraneous materials and/or that employ the signal from the labeled moiety. A number of these modifications are reviewed in, e.g., Matthews & Kricka, 1988, Anal. Biochem., 169:1; Landegren et al., 1988, Science, 242:229; Mittlin, 1989, Clinical Chem. 35:1819; U.S. Pat. No. 4,868,105, and in EPO Publication No. 225,807.

5

#### **D. Isolation of a Wild type gene**

A wild type version of a candidate gene according to the invention can be isolated by cloning from an appropriately selected genomic library according to methods well known in the art. Methods of cloning are described in Section B entitled "Production of a Polynucleotide Sequence

10 The sequence of the cloned gene will be determined by sequencing methods well known in the art (see Ausubel et al., supra and Sambrook et al., supra). Methods of sequencing employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase® (US Biochemical Corp, Cleveland, OH), Taq polymerase (Perkin Elmer, Norwalk, CT), thermostable T7 polymerase (Amersham, Chicago, IL), or combinations of recombinant polymerases and proofreading  
15 exonucleases such as the ELONGASE Amplification System (Gibco BRL, Gaithersburg, MD). Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown, MA) and the ABI 377 DNA  
20 sequencers (Perkin Elmer).

#### **E. Isolation of a Mutant Gene**

A mutant version of a candidate gene according to the invention can be isolated by cloning from an appropriately selected genomic library according to methods well known in the art. Methods of cloning are described in Section B entitled "Production of a Polynucleotide Sequence."

The sequence of the cloned gene will be determined by sequencing methods described in  
25 Section D entitled "Isolation of a Wild Type Gene."

#### **F. Identification and Characterization of Polymorphisms**

##### **a. Identification of SNPs by in silico methods (isSNPs)**

##### **1. Identification of Polymorphisms in Candidate Genes**

30 The starting point is a set of experimentally derived nucleic acid sequences. In order to be useful for SNP discovery by the invention, it is preferred that the sequences have complete chromatogram files from a gel or capillary electrophoresis sequencing machine. When this is not available, quality score data which assigns a score to each base in the sequence indicating the

likelihood of error for the basecall may be used. If neither of these data are available, the sequence may be used to assist the clustering of other sequences and in some cases to provide additional verification for a discovered SNP, but is not be used by the invention for the identification of the polymorphism.

5           The population of sequences used may constitute either a database of cDNA-derived sequences or genomic sequence. In a preferred embodiment, sequences used by the invention are from an assembled cDNA database, such as the LifeSeqGold database (Incyte Genomics, Inc(Incyte), Palo Alto, CA).

#### 10   Derivation of Nucleic Acid Sequences

cDNA was isolated from libraries constructed using RNA derived from normal and diseased human tissues and cell lines. The human tissues and cell lines used for cDNA library construction were selected from a broad range of sources to provide a diverse population of cDNAs representative of gene transcription throughout the human body. Descriptions of the human tissues and cell lines  
15   used for cDNA library construction are provided in the LIFESEQ database (Incyte Pharmaceuticals, Inc. (Incyte), Palo Alto CA). Human tissues were broadly selected from, for example, cardiovascular, dermatologic, endocrine, gastrointestinal, hematopoietic/immune system, musculoskeletal, neural, reproductive, and urologic sources.

Cell lines used for cDNA library construction were derived from, for example, leukemic cells,  
20   teratocarcinomas, neuroepitheliomas, cervical carcinoma, lung fibroblasts, and endothelial cells. Such cell lines include, for example, THP-1, Jurkat, HUVEC, hNT2, WI38, HeLa, and other cell lines commonly used and available from public depositories (American Type Culture Collection, Manassas VA). Prior to mRNA isolation, cell lines were untreated, treated with a pharmaceutical agent such as 5'-aza-2'-deoxycytidine, treated with an activating agent such as lipopolysaccharide in the case of  
25   leukocytic cell lines, or, in the case of endothelial cell lines, subjected to shear stress.

#### Sequencing of the cDNAs

Methods for DNA sequencing are well known in the art. Conventional enzymatic methods employ the Klenow fragment of DNA polymerase I, SEQUENASE DNA polymerase (U.S.

30   Biochemical Corporation, Cleveland OH), Taq polymerase (The Perkin-Elmer Corporation (Perkin-Elmer), Norwalk CT), thermostable T7 polymerase (Amersham Pharmacia Biotech, Inc. (Amersham Pharmacia Biotech), Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies Inc. (Life

Technologies), Gaithersburg MD), to extend the nucleic acid sequence from an oligonucleotide primer annealed to the DNA template of interest. Methods have been developed for the use of both single-stranded and double-stranded templates. Chain termination reaction products may be electrophoresed on urea-polyacrylamide gels and detected either by autoradiography (for radioisotope-labeled nucleotides) or by fluorescence (for fluorophore-labeled nucleotides). Automated methods for mechanized reaction preparation, sequencing, and analysis using fluorescence detection methods have been developed. Machines used to prepare cDNAs for sequencing can include the MICROLAB 2200 liquid transfer system (Hamilton Company (Hamilton), Reno NV), Peltier thermal cycler (PTC200; MJ Research, Inc. (MJ Research), Watertown MA), and ABI CATALYST 800 thermal cycler (Perkin-Elmer). Sequencing can be carried out using, for example, the ABI 373 or 377 (Perkin-Elmer) or MEGABACE 1000 (Molecular Dynamics, Inc. (Molecular Dynamics), Sunnyvale CA) DNA sequencing systems, or other automated and manual sequencing systems well known in the art.

The nucleotide sequences have been prepared by current, state-of-the-art, automated methods and, as such, may contain occasional sequencing errors or unidentified nucleotides. Such unidentified nucleotides are designated by an N. These infrequent unidentified bases do not represent a hindrance to practicing the invention for those skilled in the art. Several methods employing standard recombinant techniques may be used to correct errors and complete the missing sequence information. (See, e.g., those described in Ausubel, F.M. et al. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY; and Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY.)

#### Assembly of cDNA Sequences

Human polynucleotide sequences may be assembled using programs or algorithms well known in the art. Sequences to be assembled are related, wholly or in part, and may be derived from a single or many different transcripts. Assembly of the sequences can be performed using such programs as PHRAP (Phils Revised Assembly Program) and the GELVIEW fragment assembly system (GCG), or other methods known in the art.

Alternatively, cDNA sequences are used as “component” sequences that are assembled into “template” or “consensus” sequences as follows. Sequence chromatograms are processed, verified, and quality scores are obtained using PHRED. Raw sequences are edited using an editing pathway known as Block 1 (See, e.g., the LIFESEQ Assembled User Guide, Incyte Pharmaceuticals, Palo Alto, CA). A series of BLAST comparisons is performed and low-information segments and

repetitive elements (e.g., dinucleotide repeats, Alu repeats, etc.) are replaced by "n's", or masked, to prevent spurious matches. Mitochondrial and ribosomal RNA sequences are also removed. The processed sequences are then loaded into a relational database management system (RDMS) which assigns edited sequences to existing templates, if available. When additional sequences are added into the RDMS, a process is initiated which modifies existing templates or creates new templates from works in progress (i.e., nonfinal assembled sequences) containing queued sequences or the sequences themselves. After the new sequences have been assigned to templates, the templates can be merged into bins. If multiple templates exist in one bin, the bin can be split and the templates reannotated.

A resultant template sequence may contain either a partial or a full length open reading frame, or all or part of a genetic regulatory element. This variation is due in part to the fact that the full length cDNAs of many genes are several hundred, and sometimes several thousand, bases in length. With current technology, cDNAs comprising the coding regions of large genes cannot be cloned because of vector limitations, incomplete reverse transcription of the mRNA, or incomplete "second strand" synthesis. Template sequences may be extended to include additional contiguous sequences derived from the parent RNA transcript using a variety of methods known to those of skill in the art. Extension may thus be used to achieve the full length coding sequence of a gene.

#### Analysis of the cDNA Sequences

The cDNA sequences are analyzed using a variety of programs and algorithms which are well known in the art. (See, e.g., Ausubel, supra, Chapter 7.7; Meyers, R.A. (Ed.) (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853). These analyses comprise both reading frame determinations, e.g., based on triplet codon periodicity for particular organisms (Fickett, J.W. (1982) Nucleic Acids Res. 10:5303-5318); analyses of potential start and stop codons; and homology searches.

Computer programs known to those of skill in the art for performing computer-assisted searches for amino acid and nucleic acid sequence similarity, include, for example, Basic Local Alignment Search Tool (BLAST; Altschul, S.F. (1993) *J. Mol. Evol.* 36:290-300; Altschul, S.F. et al. (1990) *J. Mol. Biol.* 215:403-410.) BLAST is especially useful in determining exact matches and comparing two sequence fragments of arbitrary but equal lengths, whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user (Karlin, S. et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:841-845.) Using an appropriate search tool (e.g., BLAST or HMM), GenBank, SwissProt, BLOCKS, PFAM and other databases may be searched for sequences containing regions of homology to a query rbsm or RBOSM of the present

invention.

Other approaches to the identification, assembly, storage, and display of nucleotide and polypeptide sequences are provided in "Relational Database for Storing Biomolecule Information," U.S.S.N. 08/947,845, filed October 9, 1997; "Project-Based Full-Length Biomolecular Sequence Database," U.S.S.N. 08/811,758, filed March 6, 1997; and "Relational Database and System for Storing Information Relating to Biomolecular Sequences," U.S.S.N. 09/034,807, filed March 4, 1998, all of which are incorporated by reference herein in their entirety.

Protein hierarchies can be assigned to the putative encoded polypeptide based on, e.g., motif, BLAST, or biological analysis. Methods for assigning these hierarchies are described, for example, in "Database System Employing Protein Function Hierarchies for Viewing Biomolecular Sequence Data," U.S.S.N. 08/812,290, filed March 6, 1997, incorporated herein by reference.

#### Identification of Sequence Variants and Polymorphisms

The method comprise a series of filters to identify isSNPs from other sequencing variants and errors. The filters can be grouped into the following five sets of filters by the order of application in the method:

Preliminary Filters: the main filter in the first group removes the majority of base call errors by requiring a minimum phred quality score of 15. Additional filters at this stage deal with sequence alignment errors as well as errors resulting from improper trimming of vector sequence, chimeras and splice junctions.

Advanced Chromatogram Analysis: additional base call errors are then detected by examining the original chromatogram files in the vicinity of a putative SNP by an automated procedure resulting in a set of SNPs wherein the base call error rate is reduced to less than 5%.

Clone Error Filters: errors introduced during laboratory processing such as those caused by reverse transcriptase, polymerase or somatic mutation are among the most difficult to distinguish from true SNPs. The Clone Error filters use statistically generated algorithms to identify these sources of error. A small percentage of actual SNPs will be discarded at this stage.

Clustering Error Filters: these types of errors result from the incorrect clustering of close homologs, pseudo- genes or from contamination by nonhuman sequences. The filters developed to minimize these clustering errors are also statistically based. As above these filters may be reject a fraction of actual SNPs

Finishing Filters: these filters remove duplicate and redundant SNPs from the generated list of SNP, and remove SNPs which are from the hypervariable regions of hypervariable genes such as



immunoglobulin and T cell receptors.

#### Pre-processing steps

The sequences must first be trimmed to eliminate vector sequence, contamination and repetitive sequences. Then certain low information content sequences (for example, long runs of a single base, or two or three-base repeats) and repetitive sequences (for example Alu sequences in humans) must be masked (changed to N's) to prevent over-clustering errors. The clustering process then identifies the sets of sequences that are believed to be derived from the same original DNA sequence or gene. The sequences in each cluster are then aligned using a method such as phrap which also defines a consensus sequence. It will be well recognized by those skilled in the art that there are numerous existing programs for carrying out these processes, and the SNP discovery process described herein will work equally well with any of them. In the instant embodiment, the preferred processes are Blocked 1 for trimming and masking, a variety of different algorithms for clustering, and phrap for the alignment. It will be recognized by those skilled in the art that phrap and other alignment methods carry out a secondary clustering step which divides clusters into contigs, and carry out a secondary trimming step which defines the end points of the portion of each sequence which participates in the contig. The contigs then may be searched for the occurrence of SNPs.

Errors in the trimming, clustering and alignment processes will cause SNP discovery errors, usually false positives (the prediction of SNPs where they do not exist). Additional filters which are the subject of the invention are designed to recognize and remove these errors by providing the ability to identify likely errors in the processes and to correct them.

In some instances, it is preferred, as an optional step, to unmask regions of sequences which were masked because of low information content or repetitive sequence) during the clustering process can be unmasked after clustering to allow discovery of SNPs within these regions.

#### Identification of Candidate SNP Sequences

The first step in identifying candidate SNP sequences is to redefine the end points of each sequence as the points within the previous end points where a stretch of at least 10 consecutive base calls, containing at least eight base changes, matches the consensus sequence exactly. Sequence trimming errors (both at single sequence stage and at the alignment stage contribute to the false positives when foreign sequence (vector, chimera or splice variant) is similar to the real sequence and the true boundary is difficult to determine. This step is a conservative approach to avoid false positives and also filters out lower-quality sequence that the ends. The reason the length of the match

with a consensus is measured in base changes is to avoid low significance matches on repetitive sequence such as polyA.

The next step is an each position of the alignment to compare the base calls of all the aligned sequences which are between their start and end positions and which have quality scores greater than a set threshold, and which have neighboring base calls which agree with a consensus sequence and where the neighboring base calls also have a quality score > the threshold. Preferably the threshold is a phred quality score greater than or equal to 15. The possibilities are A, C, G, T, and -(deletion).

The next step is a Clone Filter where if there has been more than one base call for a sequence position, then the clone for each sequence is identified in the sequences corresponding to each clone are compared. If the base calls for different sequences from the same clone disagree, then all the sequences for this clone at this base position are removed from consideration.

After all of these filters, positions for which there is more than one base call are candidate SNPs. The "wild type" base call is the one in the consensus sequence and the others are designated candidate SNPs. If the wild type base call is a deletion, then the SNP is considered to be an insertion at the previous base.

#### Automated Chromatogram Checking

The next filters require opening of the chromatogram files for the sequences identified as containing candidate SNPs. At each candidate SNP position, the chromatogram data of each sequence passing the Identification Filters is extracted. The first step in this process utilizes a program ABIdump to translate binary ABI chromatogram files into usable form.

Multiple Base Call Algorithm filter: the ABI base calls for each sequence are compared to the phred base calls. If the base calls do not agree at the SNP position and the two adjacent flanking positions, then the sequences are removed from consideration.

Intensity Filter: if the SNP is a single base change (this step is skipped for insertions and deletions), then the process intensity values for each of four bases at the call chromatogram location of the candidate SNP base are used to compute a ratio. If we call the intensity of wild type, "wt", the intensity of the SNP base "snp", the minimum of the other two "min", and the phred quality of the base call "Q", then the wild type sequences must have

$(\text{snp} - \text{min}) < (\text{wt} - \text{min})(Q - 17)/37$  and  $Q \geq 17$  to be considered high-quality, and  $(\text{snp} - \text{min}) < (\text{wt} - \text{min})(Q - 4)/37$  and  $Q \geq 15$  to be considered a low quality pass.

The basis for these formula is that if a base is mis-called, then there is likely to be a residual peak for the correct base. The larger the peak for the wild type base, the less likely that the call of the SNP is

correct. The actual thresholds in the formula are based on empirical data from clones which were sequence multiple times and which gave a set of confirmed SNPs and error rates for algorithm optimization.

The candidate SNP passes only if at least one wild type sequence passes and at least one  
5 SNP sequence passes. The quality of the candidate SNP is the lower of the highest wild type pass level and the highest SNP pass level (if there is a high-quality wild type sequence but only low quality SNP sequences, then the candidate is low quality. A SNP quality value is returned.

#### Clone Error Quality Filters (somatic mutation/reverse transcriptase/polymerase errors)

10 The purpose of these filters is to remove errors which are actually in the clone, that is, the clone sequence was correct but the clone does not represent the individual being sequenced. Three possible sources of these errors are somatic mutations, errors made by reverse transcriptase in the process of making cDNA, and DNA polymerase errors in those situations where the DNA has been amplified by PCR at some point prior to inserting in the cloning vector. Somatic mutations can be a  
15 particular problem in sequencing clones derived from cell lines.

Polymerase errors are specific to the type of sequencing protocol used. For example, reverse transcriptase is involved in EST sequencing but not genomic clone sequencing. Polymerase is involved in the creation of extension clones (polymerase is used in all sequencing reactions, but errors are less likely to arise because only a fraction of the templates are affected in contrast to the extension  
20 process where a single polymerase product becomes a template for the entire reaction). This filter is not applied to genomic sequences in the current embodiment on the premise that the genomic sequences do not have polymerase errors, and that somatic mutations are likely to have the same profile as real SNPs.

This filter also filters out rare SNPs as well as apparent SNPs which are not real. It is  
25 difficult to determine and confirm by experiments to what extent SNP candidates are too rare to be confirmed vs. simply not real. For many applications, very rare SNPs are of less utility than common ones such that this is not a problem; however in some applications it may be advisable to turn this filter off.

#### Base change sequence analysis filter

30 The premise of this filter is that probabilities of different mutations is different depending on the source. For example true SNPs may be mostly transitions whereas reverse transcriptase mutations could be primarily G to T mutations. While this does not allow one to determine for sure

that a given change is a true SNP, it allows one to evaluate the relative likelihood that a given mutation is a true SNP. SNP confirmation data suggest that G/T SNP candidates in which there is only one clone having the T allele have a very low probability of being real SNPs. The SNP candidates are excluded from the high confidence set (they are kept in a different file-their confirmation rate is well  
5 below 50 percent). The other set which had a very low confirmation rate is any A/T SNP.

#### Frequency Filter

This filter is based on the concept that true SNPs have a different frequency profile than clone errors and that a candidate SNP which is evident in only one clone in a deep alignment is less  
10 likely to be real than one which appears in one clone in a shallow alignment. The likelihood of finding a SNP at a given sequence location is a function of the number of chromosomes sequenced. This curve is distinctly non-linear as most SNPs are sufficiently frequent, to be found with relatively few sequences. The probability of an error of this type, however is essentially linear in the number of sequences since the chance of the change occurring in two different sequences is independent. This  
15 means that the probability that a candidate SNP observed in a single clone is a true SNP is lower if the alignment is deep than if it is shallow. Any SNP occurring in a single clone in an alignment of more than 20 clones (counting only high-quality sequences which have a chance of contributing a candidate SNP) is excluded from the high confidence set.

This filter is the basis of a secondary method used to develop the base change sequence  
20 analysis filter. Comparing the set of single clone SNPs from shallow alignment's with those from deep alignment's, which are more likely to be errors, will reveal base changes which are more likely to be associated with polymerase errors and somatic mutations.

#### Clustering Error Filters

25 These filters are intended to remove candidate SNPs which result from the incorrect clustering of similar sequences such as highly homogenous genes, similar genomic sequences, and contamination from other species where the sequences of the species have been mis-labeled as human.

#### Number of base change filter

30 This filter distinguishes homologous sequences from SNPs on the basis of the frequency of variants. True SNPs occur about one per kb when comparing to sequences or once per 2 kb if the length of sequences is included, and this fraction decreases as the depth of the alignment increases.

Since EST sequences tend to be about 500 bp or less in length, then it would be expected to have not more than one SNP per four sequences. The number of SNPs in the cluster is divided by the number of sequences in the cluster and SNPs for which this number is larger than one are discarded. The higher the number, the less likely the SNP is to be real. The threshold value of one was chosen because it appears to correspond to roughly a 50 percent success rate, however the threshold value could be adjusted to higher value to accept lower confidence SNPs.

#### Distance from next polymorphism filter

This filter calculates the number of SNPs for which the sequence is the only representative within a window of 100 bases on either side, and discards any of the SNPs for which there are more than one other SNP in this window. This threshold can be set higher, but the actual fraction of SNP candidates which are true SNPs drops off to less than 50 percent.

#### Haplotype clustering filter

When sequences from different sources are inappropriately clustered, it is possible to divide them into two or more clusters which are consistent. In particular, if we take any two differences between homologs and consider the haplotypes of the clones which overlap both SNPs, there are only two haplotypes. In other words, a 2x2 matrix of haplotypes is diagonal having only two non-zero entries. If there are only two sequences, then this is expected. For each SNP, a 2x2 haplotype matrix with each other SNP is computed. If it is diagonal, and there are more than two sequences, then the sum of the diagonal elements minus one is a "cluster total" for this SNP. This "cluster total" number has proven to be empirically correlated with the confirmation rate, probably because it predicts clusters which contain para-logs, homologs and contamination from other species. Candidates SNPs which have a cluster number of less than eight are kept. This threshold value for the cluster total can be varied.

#### Redundancy/finishing filters

Redundant SNP filter: SNPs in different contigs of the same gene which have the same base change and surrounding sequence are flagged as redundant. To accommodate possible splice variants this redundancy filter also applies to SNPs which have the surrounding sequence matches on only one side.

#### T cell receptor/immunoglobulin filters

Sequences containing SNPs are filtered to remove SNPs in sequences that are homologs to T cell receptors and immunoglobulin genes because both types of genes have hyper-variable regions which could result in false positives.

## 5 Output file

SNP related data: With each candidate SNP a variety of data is kept, including the number and sources of all contributing sequences (for example gene album, HTPS, FL, WashU/Merck, etc.), the surrounding sequence, measures of the ratio and quality scores for the "best" sequence representing  
10 each allele, etc.

Sequence related data: for each sequence associated with each SNP, the following data is kept including the distance in each direction to the end of the sequence, the distance in each direction to the next base different from the consensus and passing the initial quality filters, the library, tissue ID,  
15 donor ID and comments (for example tumor, diseases, normal).

These methods have been described in patent applications entitled "Method for the Identification of Sequence Polymorphisms using Polynucleotide Sequence Databases, and Single Nucleotide Polymorphisms Identified Thereby" (Attorney Docket Nos. GX-0006 P and GX-0010 P),  
20 and are hereby incorporated by reference.

### b. Identification of polymorphisms in osteoarthritis associated genes by SSCP

The invention provides methods for detecting the presence of polymorphisms in candidate genes of the invention. The invention also provides methods for distinguishing polymorphisms which  
25 contribute to a particular disease (e.g. osteoarthritis) over polymorphisms which do not contribute to the disease.

#### 1. Identification of Polymorphisms in Candidate Genes

Identification of polymorphisms in a candidate gene, according to the invention, will involve the steps of isolating the candidate gene, determining its genomic structure and identifying polymorphisms  
30 in the DNA sequences in any portion of the entire protein-coding region. The invention also provides methods for identifying polymorphisms in the DNA sequences corresponding to RNA splice junctions. The invention also provides methods for identifying polymorphisms in the DNA sequence corresponding to the regulatory (promoter) region of the candidate gene.

A candidate gene is isolated by cloning methods well known in the art (described above). Preferably the genomic structure of a candidate gene is determined by Southern blot analysis, as described in Section C. It is expected that the entire sequence of an open reading frame (ORF) of an average entire gene can be spanned by 16 PCR-amplified DNA fragments or amplimers of an average length of 225 bp. It is expected that a smaller gene can be spanned by 1-2 amplimers and that >50 amplimers are required to span extremely large genes. Primers useful for production of the amplimers of a particular candidate gene are designed based on preexisting knowledge of the sequence of the wild type gene, according to the primer design strategies described in Section A entitled "Design and Synthesis of Oligonucleotide Primers."

For PCR amplification of a region to be tested by SSCP it is preferable to design primers that amplify overlapping regions of the candidate gene. If a sequence variation is located in a region of a candidate gene that corresponds to the region to which the primers hybridize, the primers will likely not bind, the region containing this sequence variation will not be amplified and the variation will not be detected in PCR based assays. By producing overlapping amplimers it is expected that virtually all of the sequence variations in a particular candidate gene will be detected. The amount of overlap in the amplimers is somewhat variable (approximately 20%) and the precise location of the overlapping regions will depend on the location of regions comprising a sequence that is an appropriate primer sequence. It is a possibility that a polymorphism will be located at a position just adjacent to the primer site. Consequently, sequence information will be available for only 20 bp on one side of the polymorphism and for 104-279 bp on the other side of the polymorphism. However, this should be a sufficient amount of sequence information to allow definition of a unique sequence context in which to define the particular polymorphism.

Based on screening analysis of 92 samples (184 chromosomes), it is expected that about 50% of the amplimers will demonstrate polymorphisms, and that approximately 80% of these amplimers will detect changes at single positions while the remaining 20% will detect base changes at two positions. Based on these estimates, it is expected that there will be approximately 10 sequence variations per open reading frame. However, the number of amplimers that demonstrate polymorphisms will vary depending on the number of individuals tested, the ethnicity and structure of the population being tested, and the region of DNA being tested.

Preferably, each polymorphism will be detected in the context of an SSCP fragment. Polymorphism analysis by fluorescent SSCP (fSSCP, described in detail in Section F entitled "Identification and Characterization of Polymorphisms") uses PCR to generate an amplimer of DNA to be studied. The region to be tested is defined as the region between the primers (e.g. the region that

is incorporated into the PCR product and reflects the sequence of the DNA sample being tested). The PCR primers reflect the sequence of the DNA sample being tested and are incorporated into the PCR product as one end of each strand of DNA in the PCR product. If a polymorphism occurs in a primer binding site either the PCR primer does not bind due to the mismatch and the PCR will not produce a product, or the primer binds, an amplification step occurs wherein the primer is incorporated, but the amplified product does not contain the polymorphism which occurs at the primer binding site. Therefore, fSSCP provides a method of screening a DNA sequence located between PCR primers for the presence of polymorphisms.

The sensitivity of the technique of fSSCP for detecting a polymorphism is affected by length, such that there is a substantial decrease in the detection of polymorphisms in amplimers that are greater than 300 bp in length. However, different conditions for performing SSCP at high sensitivity with larger fragments, e.g. 800-1500 bp have also been described. If the length of DNA screened per amplimer is decreased then more amplimers are required to screen a region of a given size. Therefore, efficient screening of a gene dictates that the lower limit of the size of an amplimer is 125 bp. To attain specificity for a particular gene sequence, primers are usually 20-25 bp in length, and additional criteria such as G:C content, and intra- and inter-primer complementarity are important considerations in primer design (as described above). All of these considerations are addressed if the primer3 program (Copyright (c) 1996 Whitehead Institute for Biomedical Research) is employed to design pairs of primers suitable for use in a single PCR reaction. Typically, program parameters are set so that multiple amplimers are designed in the length range of 150-300bp, with predicted primer melting temperatures in the narrow range 60-62°C. The narrow temperature range increases the likelihood that a single set of PCR conditions can be used to generate a wide variety of different amplimers.

If it is desirable to screen a contiguous stretch of DNA which is larger than the maximum fragment size desired for sensitive polymorphism detection by fSSCP (300 bp) it is necessary to use multiple amplimers (which are assayed separately) which span the region of interest. Since the primer sites in an amplimer are not tested, these sequences need to be contained within another amplimer. To test the primer sequence, overlapping amplimers are designed by an algorithm that evaluates a large number of amplimers generated by the primer3 program for the optimum overlapping set according to a cost function. Thus, a series of overlapping PCR amplification products can be used to test a contiguous stretch of DNA. Constraints on primer design are such that the absolute minimum overlap is rarely possible. As a result, some regions of overlap occur that results in 'double testing' of a particular segment of DNA. The detection efficiency is affected by the sequence context of the polymorphism; it is possible that a polymorphic site will be detected in only one of two different



amplimers which overlap the same site. One strategy that is useful for increasing polymorphism detection efficiency is to design overlapping amplimers to generate 2-fold coverage of all sequences.

SSCP does not detect 100% of polymorphisms. The invention provides for detection of polymorphisms with an efficiency of 95% under a single set of conditions using single coverage of sequences; a 2-fold screening strategy can be employed if it is necessary to increase this detection efficiency.

It is expected that the polymorphism can be located, and detected anywhere in the SSCP fragment except in the regions at each end that correspond to the sequence of the PCR primers. The precise location and identity of the sequence variation(s) of a particular SSCP fragment can be confirmed by sequencing the fragment as described in Section D entitled "Isolation of a Wild Type Gene". The sequence of a candidate gene will be compared to the known sequence of a wild-type version of the gene by using the following DNA/protein sequence analysis programs and methods.

There are a large number of freely available methods for performing sequence comparisons. These methods differ in their speed of execution, their sensitivity, and the type of comparisons they are able to make. For example one can compare two DNA sequences, two protein sequences, a DNA sequence to a protein sequence by conceptual translation, or DNA sequences as if they were protein sequences, again by conceptual translation. The BLAST suite of programs (Altschul et al., 1990, J.Mol.Biol. 215:403) are commonly used to perform the above-referenced type of analysis. Although the BLAST suite of programs provides a rapid method of determining multiple distinct similarities between two sequences, these programs are not guaranteed to find an optimal solution when comparing two sequences according to a particular set of parameters. PSI-BLAST is a more sensitive variant of BLAST that operates by iteratively searching the database while simultaneously refining the query pattern based on the results of the searches. Other packages of programs that are available and which have different specific properties include the HMMER, SAM, WISE, STADEN and FASTA packages, and the programs est\_genome, dotter, e-PCR, Clustal, cross\_match and phrap (Pearson, 1996, Methods Enzymol., 266:227).

If sequence information is available for the intron-exon boundaries and for a region of the intron (of approximately 30-150 bp) located immediately 5' of an intron-exon boundary, primers can be designed to produce amplimers useful for identifying polymorphisms located in the RNA splice junctions. Similarly, if the promoter region of a candidate gene has been sequenced, primers can be designed to produce amplimers useful for identifying polymorphisms located in the promoter region. Additional methods for detecting and isolating polymorphisms include, but are not limited to fluorescent polarization-TDI, mass spectroscopy denaturing gradient gel electrophoresis, chemical cleavage of

mismatch, constant denaturant capillary electrophoresis, RNase cleavage, heteroduplex analysis, sequencing by hybridization, DNA sequencing, representational difference analysis, and denaturing high performance liquid chromatography, described below in Section F entitled, "Identification and Characterization of Polymorphisms".

5

## 2. Methods of Determining if a Polymorphism Contributes to osteoarthritis

No two individuals (excluding identical twins or other clones) have the same sequence of DNA in their genome. Variability in gene sequences between individuals accounts for many of the obvious phenotypic differences (such as pigmentation of hair, skin, etc.) and many nonobvious ones (such as drug tolerance and disease susceptibility). In a population, the DNA sequence that occurs at the highest frequency at any given site is commonly referred to as the wild type sequence. The term "wild type sequence" can be misleading, however, because in different populations an alternative form of a DNA sequence may be predominant and thus considered wild type for that particular population. DNA polymorphisms are located throughout the genome, within and between genes, and the various forms may or may not result in differential gene function (as determined by comparing the function of two alternative forms of the same sequence). Most polymorphisms do not alter gene function and are called neutral polymorphisms. Some polymorphisms do have an effect on gene function, for example by changing the amino acid sequence of a protein, or by altering control sequences such as promoters or RNA splicing or degradation signals.

Polymorphisms can be used in genetic studies to identify a gene involved in a disease. If a polymorphism alters a gene function such that it increases disease susceptibility, then it will be present more often in individuals with the disease than in those without the disease. Alternatively, if a particular DNA variant is protective against a disease, it will be found more often in individuals without the disease than in those with the disease. Statistical methods are used to evaluate polymorphism frequencies found in diseased as compared to normal populations, and provide a means for establishing a causal link between a polymorphism and a phenotype. To detect a significant association between a disease and a polymorphic site, different tests may be used with either genotypic or allelic distributions. The simplest test consists of a t-test wherein the frequency of the polymorphic alleles in normal individuals and individuals with the disease phenotype is compared. A comparison of the genotypic distribution in normal individuals and individuals with the disease phenotype can also be performed using a chi-square test of homogeneity. These tests are implemented in all commercially or freely available statistical packages, for example SAS and S+, and are even included in Microsoft Excel. More sophisticated analyses will be performed by incorporating covariates such as linear regression or

logistic regression, and by accounting for the information provided by adjacent polymorphic sites (multipoint analysis). An example of this type of program is the freely available program "Analyze" by JD Terwilliger (currently available at the WWW site <ftp://ftp.well.ox.ac.uk/pub/genetics/analyze>).

If a polymorphism has a phenotypic effect, a bias will exist in the distribution of polymorphisms between groups that have and do not have the disease phenotype. This manner of analysis can be used to study a trait that is not necessarily a disease; any trait can be studied by comparing a group with a particular phenotypic form of a trait to a group with a different phenotypic form of that trait. It is important that the cases and controls are correctly matched with regards to ethnicity, environmental influences, and other factors which could effect the phenotype being studied. Studies which test polymorphism frequencies within groups exhibiting different phenotypes and use statistical methods to compare the group polymorphism frequencies and identify correlations with phenotypes, are known as "associations studies".

Some polymorphisms that occur in a single gene can alter the function of a gene sufficiently such that the polymorphism results in a disease (monogenic disease). However, many common human diseases are polygenic; that is they are the result of complex interactions of various forms of multiple genes. In the case of polygenic diseases, the alteration of a single gene may not be detrimental per se, but in combination with certain sequence variants of other genes, this altered DNA sequence may contribute to a disease phenotype. DNA variants leading to monogenic diseases are usually rare in a population due to the process of natural selection against those carrying the disease gene. As variants in genes that are involved in polygenic disease do not produce the disease phenotype unless they occur in the appropriate combination with other gene variants, normal individuals can carry a subset of the disease-contributing variants without suffering adverse effects. Thus, disease-contributing gene variants that are associated with polygenic diseases may exist at a high frequency in a normal population. Selection against these disease variant forms of a gene will only occur when they are present in the appropriate disease-causing combination and there may not necessarily be selection against these gene variants in individuals carrying a subset of the disease-contributing variants. Neutral DNA variants do not alter gene function or contribute to a disease, are under no selective pressure and occur at variable frequencies within populations.

Monogenic diseases tend to be rare within the population, and therefore few patients may be available for studies of these diseases. A polymorphism in a single specific gene is necessary and usually sufficient to cause a monogenic disease, such that associations between the variant gene and the phenotype are usually readily apparent. In cases where the expression of a mutation phenotype is complete, ("complete penetrance"), the polymorphism present in the disease gene will not be found

upon examination of a large number of normal individuals. If there is not complete penetrance then some apparently normal individuals will contain the mutation; the difference in frequency of occurrence of the variant gene in the disease group as compared to the normal population will reveal that the variant is associated with the disease.

5           In polygenic diseases, variation at different genes occurs in a combination which alters susceptibility to the disease. Although several genes may have variant forms which can contribute to a disease phenotype, it is not always necessary for a contributing variant to be present at every gene potentially contributing to the disease in a given affected individual. For example, a hypothetical disease could be caused by a particular combination of variants at three of four genes, designated as  
10   A, B, C, and D. Appropriate susceptibility variants in combination at any three of the genes can cause the susceptibility, i.e. one person with increased susceptibility may have susceptibility variants in genes A, B, and C, while another individual with increased susceptibility to the same disease will have susceptibility variants in genes B, C, and D. Therefore, although not all affected individuals will have the same susceptibility variants, the net result is that a diseased population will have susceptibility  
15   variant forms of genes A, B, C, and D at a higher frequency than an unaffected population (as detected by association studies).

          Unlike monogenic diseases which result from polymorphisms that are not present in control  
populations, the polymorphisms which contribute to the polygenic disease are also present in a normal population. As described in the example above, an individual with susceptibility polymorphisms in only  
20   one or two of the genes potentially contributing to the disease susceptibility will be normal with regard to disease susceptibility. Therefore, normal populations can be used to identify polymorphic regions of the genome in the population, and these regions can then be specifically tested in larger patient and control populations. Typically, a gene is analyzed for the presence of polymorphisms by testing between 2 and 100 normal individuals in order to establish if a particular polymorphism is present for  
25   that gene in the population. Once a polymorphic site(s) has been defined, the polymorphic site is then tested in case (disease) and control (normal) populations and statistical analyses are performed to identify polymorphisms which occur at significantly different frequencies in the two populations.

          The determination of the statistical significance of polymorphism frequency differences is dependent upon the size of the observed frequency difference between the populations, and on the  
30   size of the populations being studied. If a significant difference is found, then it can be concluded that an association exists between the polymorphism and the phenotype being studied. A statistically significant difference is a frequency difference at a particular site between populations which would be expected to occur by chance in only 5 out of 100 tests. That is, a difference which has a 95%

probability of being a true difference due to the affect of the gene.

The foregoing discussion describes a method of testing for an association between a polymorphism which is the direct contributor to a disease and the disease phenotype. However, polymorphisms which do not directly contribute to a disease can also be used to identify regions of the genome which contain genes that contribute to the disease by virtue of their proximity to disease-contributing polymorphisms.

In humans, DNA exists as 23 homologous pairs of linear molecules (chromosomes). Recombination is a process which results in reciprocal exchanges of short homologous DNA segments between these homologous DNA pairs. Only one of each of the 23 pairs of chromosomes is inherited by the offspring. The inherited chromosome is thus made up of tandemly arrayed segments of DNA derived from both of a pair of chromosomes. Consequently, DNA is transferred in segments from one generation to the next. Although the boundaries of each inherited segment may vary in each generation, the net effect is that sequences of DNA which are adjacent along the length of the molecule are inherited together at a higher frequency than sequences that are farther apart. If a region (continuous linear segment) of DNA has two or more polymorphisms that are close together, they will be co-inherited at a higher frequency than polymorphisms that are farther apart, as they are more likely to remain on the same segment of DNA during recombination. Therefore, if two or more polymorphisms are close together, they will occur together at a higher frequency in a population than would be expected by random segregation. This effect is known as linkage. Linkage studies are performed using multiply affected individuals within families; the most commonly used approach is to test markers located throughout the genome in many sets of affected sib pairs that share the same phenotype. Markers which are located in the region of a genome that contributes to the phenotype will be inherited in both siblings, along with the phenotype, at a higher frequency than expected by chance. Studies wherein data from many such families is compared can be used to implicate a region of a genome as one that contributes to a particular phenotype.

Linkage disequilibrium (LD) association studies provide another method for using polymorphisms in genetic studies. The method of LD involves making a correlation at the population level, between the alleles (alternative polymorphic forms of the same sequence site) present at different genomic sites. If site 1 has two variant forms, A and a, and site 2 has two variant forms B and b, the observation in a population that allele A at site 1 is more often found with allele B at locus 2 than with allele b is an example of LD. If allele B is a disease-contributing polymorphism, then testing at allele A may show an association with the disease.

Linkage disequilibrium may be generated in several ways. Maintenance of LD in a population

allows a disease association to be detected many generations after the formation of LD. The maintenance of LD is explained by linkage: the closer the two loci, the longer (in terms of number of generations) that particular LD is maintained. As a result, polymorphisms which do not directly contribute to a disease can be used to identify regions of the genome which contain a disease contributing polymorphism. If a polymorphism affects gene function such that it contributes to a phenotype being studied and is found to be associated with the phenotype, nearby (neutral) polymorphisms which are in LD with the disease polymorphism may also show an association with the disease. Conversely, if a polymorphism does not affect gene function but is found to be associated with a particular phenotype, this polymorphism is in LD with a different, but adjacent polymorphism that affects gene function such that it contributes to the phenotype being studied. If a neutral polymorphism is always inherited with a phenotype- contributing polymorphism, then the strength of the association of the neutral polymorphism to the phenotype will be equal to that of the polymorphism which affects gene function and is contributing to the phenotype. A polymorphism which shows an association with a phenotype (for instance with disease susceptibility) is a marker for that phenotype and implicates the region in which the polymorphism resides as a region containing a polymorphism which contributes to the phenotype. Additional flanking polymorphisms can be tested to determine the precise location of the true phenotype-contributing variant.

Linkage studies on families, and LD studies on populations have different degrees of resolution with regards to defining the size of a DNA region which contains the phenotype-contributing polymorphism. In general, linkage studies define an interval which potentially contains tens to hundreds of genes, while LD studies have been used to implicate single genes in the development of a particular phenotype.

### 3. Test Populations Useful for Polymorphism Genotyping

The invention provides methods of determining allelic frequencies by performing genotypic analyses in appropriate test populations.

Study cohorts:

#### Osteoarthritis Progression Cohort

Derived from a population of normal women aged 45-65. The original aims of the study, started in 1989, were to assess how many women around menopausal age would get arthritis and what factors predispose them to developing it. Also to look into factors that may be associated with progression of the disease.

A series of examinations, x-rays and questionnaires about lifestyle factors were carried out on 1003 women that were recruited to the study. This study has been going for 10 years. As a result, a unique, world-renowned and well respected study is available looking at the reasons why women develop osteoarthritis, potential risk factors and the genetics of the disease.

#### Prospective Severe Outcomes Cohort (case-control)

Five hundred joint replacement cases will be ascertained as will be age, ethnicity and gender matched controls. The clinical data envisaged are : HRT use, numbers of joints affected, occupation, injury history, age, BMI.

The list of studies relevant is shown in following table.

<i>Study type</i>	<i>Population details</i>	<i>Reasonable objectives</i>	<i>Timing</i>
Pilot <sup>1</sup>	100 progressors + 75 non-progressors, 100 normals, all female from the progression cohort. Detailed clinical data, 10 yr. follow-up: joint-space narrowing/yr., joints affected, BMD, fractures, CRP levels.	Large genetic effects for fast OA progression, proof of principle. Correlation with biomarkers. Possible novel target.	6-8 Months
Biomarker study	800 women from progression cohort. DNA, serum, urine. 5 biomarkers	Correlation of genetics with biomarkers - v. useful for clinical trials.	12 Months
Progression hand & knee OA study	~800 women from progression cohort. Detailed clinical data, joint-space narrowing/yr., joints affected, BMD (hip and spine), fractures, CRP levels, full lipid measurements, incidence of fractures (assessed by X-rays), 10 yr. follow-up radiographs for all patients.	Genetic effects of OA progression. Risk of OA. Correlation with biomarkers. Possible novel target. Genetic effects of osteoporosis risk, correlation with BMD. Possibly genetic effects of lipid levels and CVD risk.	18 Months
Case-control	~500 cases (joint replacements) Vs 500 matched controls. Prospective study: DNA + 2 biomarkers. Clinical data required: steroid use, #joints affected,	Large genetic effects for OA risk, proof of principle. Possible novel target.	~6-12 months for collection



occupation, injury history, age, BMI

#### 4. Assays Useful for Determining the Association of a Polymorphism with osteoarthritis

##### Clinical parameters

There is a general consensus that radiological changes are the preferred method for epidemiological studies on the basis of cross sectional and prospective correlations between severity of X-ray changes with the presence of pain and loss of function. In osteoarthritis, the loss of cartilage produces a narrowed space between bones. The pattern of joint space narrowing can help distinguish between osteoarthritis and rheumatoid arthritis. Bone spurs (osteophytes) also help diagnose osteoarthritis. Other relevant clinical end points are pain, disability, function, joint replacement and maintenance of joint structure. Stages of disease progression are as follows:

Early stage: focal swelling of articular cartilage followed by the appearance of irregularities in the surface.

Intermediate stage: progressive degradation and loss of articular cartilage. Also characterised by fibrillation (vertical splitting), detachment (horizontal splitting) and thinning of the cartilage.

Late stage: Articular cartilage is almost completely destroyed. Bony outgrowths (osteophytes) occur at the joint margins resulting in residual arthritis. Characterised by pain and limitation of joint movement.

##### Clinical measurements of OA

Quantitative traits of interest for the study of OA and its progression are:

- Osteophyte count.
- Joint space narrowing (mm/yr.)
- Number of joints affected
- Types of joints affected



In addition a series of biochemical markers can provide valuable information such as:

- COMP
- CRP
- 5 - HA
- Protocollagen Type II
- Bone resorption markers (e.g. collagen cross-links)

#### Confounding factors

10 Most currently recognised environmental risk factors for prevalent knee OA - obesity, knee injury, and physical activity, influence incidence more than radiographic progression. Furthermore, these factors might selectively influence osteophyte formation more than joint space narrowing. These findings are consistent with knee OA being initiated by joint injury, but with progression being a consequence of impaired intrinsic repair capacity.

15 Other known confounding factors are steroid (glucocorticoid) use and, in women, hormone replacement therapy. Glucocorticoids ameliorate erosion in animal OA models and suppress synthesis of matrix metalloproteinases (Saito et al. 1999). Estrogen replacement therapy, on the other hand, has been shown to have a moderate, but not statistically significant, protective effect against worsening of OA both in the Chingford (Hart et al. 1999) and Framingham (Zhang et al. 1998) studies.

20

#### 5. Methods of Genotyping Polymorphisms

The invention discloses methods for performing polymorphism genotyping. These methods can be used to detect the presence of a polymorphism in a sample comprising DNA or RNA.

25 A DNA sample for analysis according to the invention may be prepared from any tissue or cell line, and preparative procedures are well-known in the art. The preparation of genomic DNA is performed as described in Section B.

RNA samples may also be useful for genotyping according to the invention. Isolation of RNA can be performed according to the following methods.

30 RNA is purified from mammalian tissue according to the following method. Following removal of the tissue of interest, pieces of tissue of  $\leq 2$ g are cut and quick frozen in liquid nitrogen, to prevent degradation of RNA. Upon the addition of a volume of 20 ml tissue guanidinium solution per 2 g of tissue, tissue samples are ground in a tissuemizer with two or three 10-second bursts. To prepare tissue guanidinium solution (1 L) 590.8 g guanidinium isothiocyanate is dissolved in approximately 400

ml DEPC-treated H<sub>2</sub>O. 25 ml of 2 M Tris-Cl, pH 7.5 (0.05 M final) and 20 ml Na<sub>2</sub>EDTA (0.01 M final) is added, the solution is stirred overnight, the volume is adjusted to 950 ml, and 50 ml 2-ME is added.

Homogenized tissue samples are subjected to centrifugation for 10 min at 12,000 x g at 12°C.

5 The resulting supernatant is incubated for 2 min at 65°C in the presence of 0.1 volume of 20% Sarkosyl, layered over 9 ml of a 5.7M CsCl solution (0.1g CsCl/ml), and separated by centrifugation overnight at 113,000 x g at 22°C. After careful removal of the supernatant, the tube is inverted and drained. The bottom of the tube (containing the RNA pellet) is placed in a 50 ml plastic tube and incubated overnight (or longer) at 4°C in the presence of 3 ml tissue resuspension buffer (5 mM  
10 EDTA, 0.5% (v/v) Sarkosyl, 5% (v/v) 2-ME) to allow complete resuspension of the RNA pellet. The resulting RNA solution is extracted sequentially with 25:24:1 phenol/chloroform/isoamyl alcohol, followed by 24:1 chloroform/isoamyl alcohol, precipitated by the addition of 3 M sodium acetate, pH 5.2, and 2.5 volumes of 100% ethanol, and resuspended in DEPC water (Chirgwin et al., 1979, Biochemistry, 18: 5294).

15 Alternatively, RNA is isolated from mammalian tissue according to the following single step protocol. The tissue of interest is prepared by homogenization in a glass teflon homogenizer in 1 ml denaturing solution (4M guanidinium thiocyanate, 25 mM sodium citrate, pH 7.0, 0.1 M 2-ME, 0.5% (w/v) N-laurylsarkosine) per 100mg tissue. Following transfer of the homogenate to a 5-ml polypropylene tube, 0.1 ml of 2 M sodium acetate, pH 4, 1 ml water-saturated phenol, and 0.2 ml of  
20 49:1 chloroform/isoamyl alcohol are added sequentially. The sample is mixed after the addition of each component, and incubated for 15 min at 0-4°C after all components have been added. The sample is separated by centrifugation for 20 min at 10,000 x g, 4°C, precipitated by the addition of 1 ml of 100% isopropanol, incubated for 30 minutes at -20°C and pelleted by centrifugation for 10 minutes at 10,000 x g, 4°C. The resulting RNA pellet is dissolved in 0.3 ml denaturing solution, transferred to a  
25 microfuge tube, precipitated by the addition of 0.3 ml of 100% isopropanol for 30 minutes at -20°C, and centrifuged for 10 minutes at 10,000 x g at 4°C. The RNA pellet is washed in 70% ethanol, dried, and resuspended in 100-200 ml DEPC-treated water or DEPC-treated 0.5% SDS (Chomczynski and Sacchi, 1987, Anal. Biochem., 162: 156).

RNA prepared according to either of these methods can be used for genotyping by the  
30 methods of Northern blot analysis, S1 nuclease analysis and primer extension analysis (Ausubel et al., supra).

cDNA samples also may be prepared according to the invention, i.e., DNA that is complementary to RNA such as mRNA. The preparation of cDNA is well-known and well-

documented in the prior art.

cDNA is prepared according to the following method. Total cellular RNA is isolated (as described) and passed through a column of oligo(dT)-cellulose to isolate polyA RNA. The bound polyA mRNAs are eluted from the column with a low ionic strength buffer. To produce cDNA molecules, short deoxythymidine oligonucleotides (12-20 nucleotides) are hybridized to the polyA tails to be used as primers for reverse transcriptase, an enzyme that uses RNA as a template for DNA synthesis. Alternatively, mRNA species can be primed from many positions by using short oligonucleotide fragments comprising numerous sequences complementary to the mRNA of interest as primers for cDNA synthesis. The resultant RNA-DNA hybrid can be converted to a double stranded DNA molecule by a variety of enzymatic steps well-known in the art (Watson et al., 1992, Recombinant DNA, 2nd edition, Scientific American Books, New York).

Tissues or fluids which are useful for obtaining a DNA or RNA sample according to the invention include but are not limited to plasma, serum, spinal fluid, lymph fluid, external secretions of the skin, respiratory, intestinal and genitourinary tracts, saliva, blood cells, tumors, organs, tissue and samples of *in vitro* cell culture constituents.

Genotyping methods which are useful according to the invention, i.e., for the detection of polymorphisms in nucleic acid samples isolated from individuals, are disclosed below.

#### Single Strand Conformation Polymorphism (SSCP) Screening and Fluorescent SSCP Screening (fSSCP)

##### SSCP Analysis

One technique for detecting DNA sequence variations in a biological sample is single strand conformation polymorphism (SSCP) (Glavac et al., 1993, Hum. Mut. 2:404; Sheffield et al., 1993, Genomics 16:325). SSCP is a simple and effective technique for the detection of single base changes. This technique is based on the principle that single-stranded DNA molecules assume specific sequence-based secondary structures (conformers) under nondenaturing conditions. The detection of point mutations by single stranded conformation polymorphism is believed to be due to an alteration in the structure of single stranded DNA. Molecules differing by only a single base substitution may assume different conformers and migrate differently in a nondenaturing polyacrylamide gel. Single stranded DNAs that contain sequence variations are identified by an abnormal mobility on polyacrylamide gels. SSCP detects all types of point mutations and short insertions or deletions that are located between the PCR primers (within the probe region) with apparently equal efficiency. This

technique has proven useful for detection of multiple mutations and polymorphisms, including SNPs. SSCP sensitivity varies dramatically with the size of the DNA fragment being analyzed. The optimal size fragment for sensitive detection by SSCP is approximately 125-300bp.

The mobility of a single stranded DNA or double stranded DNA fragment during electrophoresis through a gel matrix is dependent on its size. Small molecules migrate more rapidly than large molecules because they pass through the pores in the matrix more easily. Conventionally, electrophoresis of single stranded DNA involves a 'denaturing' gel which maintains the single strandedness of the molecules. The denaturant is typically urea in polyacrylamide gels, and typically formamide or sodium hydroxide in agarose gels. In contrast, according to the SSCP screening protocol, single-stranded DNA is analyzed on a 'nondenaturing' gel. When single stranded DNA is analyzed on a 'non-denaturing' gel, intramolecular interactions can occur. In particular, the single stranded DNA is able to (partially) bind to itself. Consequently, DNA that is separated by electrophoresis on an SSCP gel does not migrate as a linear molecule but rather, the mobility of the DNA on an SSCP gel is governed by both its size and tertiary structure (conformation). The tertiary structure of a single stranded DNA fragment is dependent on the sequence of the entire fragment. Therefore, if a polymorphism exists in a given fragment, the conformation will usually be altered. The technique is performed as follows.

One or more test DNA samples are prepared for analysis as described above, and subject to PCR amplification. Oligonucleotide primers are designed and synthesized as described above.

Amplifications are performed in a total volume of 10 ml containing 50 mM KCl, 10 mM Tris-HCl, pH 9.0 (at 25°C), 0.1 % Triton X-100, 1.5 mM MgCl<sub>2</sub>, 0.2mM of dGTP, dATP, dTTP, 0.02 mM of non radioactive dCTP, 0.05 ml [ $\alpha$ -<sup>33</sup>P] dCTP (1,000-3,000 Ci mmol<sup>-1</sup>; 10 mCi ml<sup>-1</sup>), 0.2 uM each primer, 50 ng genomic DNA (or 1 ng of cloned DNA template) and 0.1 U Taq DNA polymerase. The PCR cycling profile is as follows : preheating to 94°C for 3 min followed by 94°C, 1 min; annealing temperature, 30 sec; 72°C, 45 sec for 35 cycles and a final extension at 72°C for 5 min. Annealing temperature is different for each PCR primer pair and can be optimized according to the parameters described above. Amplifications using Vent Taq polymerase (New England Biolabs) are performed in a total volume of 10 ul using the buffer provided by the manufacturer with 1 mM each of dGTP, dATP, dTTP, 0.02 mM dCTP, 0.25 ul [ $\alpha$ -<sup>33</sup>P] dCTP (1,000-3,000 Ci mmol<sup>-1</sup>; 10 mCi ml<sup>-1</sup>), 0.2 uM of each primer, 50 ng of genomic DNA (or 1 ng of cloned DNA template) and 0.1 U of Vent Taq DNA polymerase. Samples are heated to 98°C for 5 min prior to addition of enzyme and nucleotides. The PCR cycling profile is 98°C, 1 min; annealing temperature, 45 sec; 72°C, 1 min for 35 cycles, followed by a final extension at 72°C for 5 min. The length and temperature of each step of a PCR cycle, as

well as the number of cycles, is adjusted in accordance to the stringency requirements, as described above.

SSCP analysis is performed as follows. Ten ul of formamide dye (95% formamide, 20mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol) are added to 10 ul aliquots of radiolabeled PCR product. Following denaturation at 100°C for 5 min, the reaction mixture is placed on ice. Two ul aliquots are loaded onto 8% acrylamide:bisacrylamide (37.5:1), 0.5X TBE (45 mM Tris-borate, 1 mM EDTA), 5% glycerol gels. Electrophoresis is carried out at 25W at 4°C for 8 hours in 0.5X TBE. Dried gels are exposed to X-OMAT ARfilm (Kodak) and the autoradiographs are analyzed and scored for aberrant migration of bands (band shifts). SSCP may be optimized, as desired, as taught in Glavac et al., 1993, Hum. Mut. 2:404.

#### fSSCP Analysis

Techniques for screening multiple DNA samples simultaneously are also useful for performing rapid genotyping analysis on a large number of samples according to the invention. By pooling and multiplexing DNA samples in fluorescent SSCP (fSSCP) assays, the high throughput required for detecting sequence variations in a large number of samples is achieved (Makino et al., 1992, PCR Methods Appl. 2:10; Ellison et al., 1993, BioTechniques 15:684). According to the method of fSSCP, PCR products are visualized and analyzed using an ABI fluorescent DNA sequencing machine. Different primer pairs are identified by different color fluorochromes (4 different fluorochromes are now available). fSSCP offers the following advantages over SSCP. Unlike SSCP, fSSCP does not require handling of radioactive materials. Furthermore, the fSSCP technique allows for automated data and automated data analysis programs that detect aberrantly migrating samples. In contrast, SSCP evaluation involves visual examination by an individual, and does not provide a means for correcting for lane to lane variations in electrophoretic conditions, as does fSSCP analysis.

fSSCP Analysis is performed as follows.

Amplifications are performed in a total volume of 10 ul containing 50 mM KCl, 10mM Tris-HCl, pH 9.0 (at 25 °C), 0.1 % Triton X-100, 1.5 mM MgCl<sub>2</sub>, 0.2mM of dGTP, dATP, dTTP, dCTP, 0.2 uM primer labeled with one of the fluorochromes HEX, FAM, TET or JOE, 50 ng genomic DNA (or 1 ng of cloned DNA template) and 0.1 U Taq DNA polymerase. The PCR cycling profile is as follows : preheating to 94°C for 3 min followed by 94°C, 1 min; annealing temperature, 30 sec; 72°C, 45 sec for 35 cycles and a final extension at 72°C for 5 min. Annealing temperature is different for each PCR primer pair. Amplifications using Vent Taq polymerase (New England Biolabs) are performed in a total volume of 10 ul using the buffer provided by the manufacturer with 1 mM each of

dGTP, dATP, dTTP, dCTP, 0.2 uM primer labeled with one of the fluorochromes HEX, FAM, TET or JOE, 50 ng genomic DNA (or 1 ng of cloned DNA template) and 0.1 U of Vent Taq DNA polymerase. Samples are heated to 98°C for 5 min prior to addition of enzyme and nucleotides. The PCR cycling profile is 98°C, 1 min; annealing temperature, 45 sec; 72°C, 1 min for 35 cycles, followed by a final extension at 72°C for 5 min. Annealing temperature is different for each PCR primer pair. Two ul of fluorescent PCR products are added to 3 ul formamide dye (95% formamide, 20mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol), denatured at 100°C for 5 min, then placed on ice. Thereafter, 0.5-1 ml of Genescan™ 1500 size markers are added as an internal standard. Two ul of the mix is loaded onto 8% or 10% acrylamide:bisacrylamide (37.5:1), 0.5X TBE (45 mM Tris-borate, 1 mM EDTA), 5% glycerol gels and electrophoresis is performed on an ABI 377 DNA sequencing machine. Gel temperature is maintained between 4° and 10°C by an external cooling unit connected to the internal cooling plumbing and chambers. Electrophoresis is carried out at 2500-3500 volts for 4 - 10 hours in 0.5X TBE. Data is automatically collected and analyzed with Genescan and Genotype analysis software (ABI).

The fSSCP procedure identifies regions of 150-300 base pairs containing a sequence variation. To identify the exact sequence change, the fragment which demonstrates the aberrant migration is amplified again from the same biological sample, using non fluorescent primers. The sequence is then determined using standard DNA sequencing methods well known to those skilled in the art (Ausubel et al., supra).

Although SSCP and fSSCP techniques are preferred according to the invention, other methods for detecting sequence variations, including DNA sequencing, can be employed. Additional techniques for detecting DNA sequence variations useful according to the invention are described below.

#### Fluorescence Polarization-TDI

Fluorescence polarization-TDI is another preferred technique according to the invention for the detection of sequence variations. Template-directed primer extension is a dideoxy chain terminating DNA sequencing protocol designed to ascertain the nature of the one base immediately 3' to the sequencing primer that is annealed to the target DNA immediately upstream from the polymorphic site. In the presence of DNA polymerase and the appropriate dideoxynucleoside triphosphate (ddNTP), the primer is extended specifically by one base as dictated by the target DNA sequence at the polymorphic site. By determining which ddNTP is incorporated, the alleles present in the target DNA can be determined.

Fluorescence polarization is based on the observation that when a fluorescent molecule is excited by plane-polarized light, it emits polarized fluorescent light into a fixed plane if the molecules remain stationary between excitation and emission. However, because the molecule rotates and tumbles in solution, fluorescence polarization is not observed fully by an external detector. The fluorescence polarization of a molecule is proportional to the molecule's rotational relaxation time, which is related to the viscosity of the solvent, absolute temperature, molecular volume, and the gas constant. If the viscosity and temperature are held constant, then fluorescence polarization is directly proportional to the molecular volume, which is directly proportional to the molecular weight. If the fluorescent molecule is large (with high molecular weight), it rotates and tumbles more slowly in solution and fluorescence polarization is preserved. If the molecule is small (with low molecular weight), it rotates and tumbles faster and fluorescence polarization is largely lost (depolarized).

In the FP-TDI assay, the sequencing primer is an unmodified primer with its 3' end immediately upstream from a polymorphic or mutation site. When incubated in the presence of ddNTPs labeled with different fluorophores, the allele-specific dye ddNTP is incorporated onto the TDI primer in the presence of DNA polymerase and target DNA. The genotype of the target DNA molecule can be determined simply by exciting the fluorescent dye in the reaction and determining whether a change in fluorescence polarization occurs. Chen et al., 1999, *Genome Res.*, 9:492.

One or more test DNA samples are prepared for analysis as described above, and subject to PCR amplification. Oligonucleotide primers are designed and synthesized as described above. Amplifications are performed in a total volume of 10 ml containing 50 mM KCl, 10 mM Tris-HCl, pH 9.0 (at 25°C), 0.1 % Triton X-100, 1.5 mM MgCl<sub>2</sub>, 0.2mM of dGTP, dATP, dTTP, 0.02 mM of non radioactive dCTP, 0.05 ml [<sup>33</sup>P] dCTP (1,000-3,000 Ci mmol<sup>-1</sup>; 10 mCi ml<sup>-1</sup>), 0.2 uM each primer, 50 ng genomic DNA (or 1 ng of cloned DNA template) and 0.1 U Taq DNA polymerase. The PCR cycling profile is as follows : preheating to 94°C for 3 min followed by 94°C, 1 min; annealing temperature, 30 sec; 72°C, 45 sec for 35 cycles and a final extension at 72°C for 5 min. Annealing temperature is different for each PCR primer pair and can be optimized according to the parameters described above. Amplifications using Vent Taq polymerase (New England Biolabs) are performed in a total volume of 10 ul using the buffer provided by the manufacturer with 1 mM each of dGTP, dATP, dTTP, 0.02 mM dCTP, 0.25 ul [<sup>33</sup>P] dCTP (1,000-3,000 Ci mmol<sup>-1</sup>; 10 mCi ml<sup>-1</sup>), 0.2 uM of each primer, 50 ng of genomic DNA (or 1 ng of cloned DNA template) and 0.1 U of Vent Taq DNA polymerase. Samples are heated to 98°C for 5 min prior to addition of enzyme and nucleotides. The PCR cycling profile is 98°C, 1 min; annealing temperature, 45 sec; 72°C, 1 min for 35 cycles, followed by a final extension at 72°C for 5 min. The length and temperature of each step of a PCR cycle, as

well as the number of cycles, is adjusted in accordance to the stringency requirements, as described above.

Following PCR amplification, unused PCR primers and dNTPs are destroyed by adding 2ml of PCR product to 2ml of SAP/Exonuclease cocktail (0.1U shrimp alkaline phosphatase (1 U/ml, Amersham Pharmacia Biotech, Inc., Piscataway, NJ) and 0.2U *E. coli* exonuclease I (10 U/ml, Amersham) in SAP buffer (20mM TrisHCl, pH 8.0; 10 mM MgCl<sub>2</sub>, Amersham)) per well of a 384-well Black PCR plate (ABT). The mixtures are incubated at 37°C for 60 min before the enzymes are heat inactivated at 95°C for 15 min. The mixture is held at 4°C until used in the FP-TDI assay.

To the enzymatically treated PCR product, 2 ml of TDI reaction cocktail containing TDI buffer (50mM Tris-HCl (pH 9.0), 50mM KCl, 5 mM NaCl, 2 mM MgCl<sub>2</sub>, 8% glycerol), 1 mM TDI primer, 12.5 nM of each of two allele specific dye-labeled ddNTPs (ROX-ddGTP, BFL-ddATP, Tamra-ddCTP, or R6G-ddUTP; NEN Life Science Products, Inc., Boston, MA), and 0.32U Thermo Sequenase (Amersham). The reaction mixtures are incubated at 94°C for 15 min, followed by 34 cycles of 94°C for 30 seconds and 55°C for 15 seconds. Upon completion of the reaction cycles, the samples are held at 4°C.

After the primer extension reaction, 24 ml of TE buffer/methanol (2:1) is added to each sample well, and the fluorescence polarization is measured using a LJI Analyst (LJI Biosystems, Sunnyvale, CA).

## Denaturing Gradient Gel Electrophoresis

Denaturing gradient gel electrophoresis (DGGE) is a gel system which allows electrophoretic separation of DNA fragments differing in sequence by a single base pair. The separation is based upon differences in the temperature of strand dissociation of the wild-type and mutant molecules. During electrophoresis, fragments migrating through the gel are exposed to an increasing concentration of denaturant in the gel. When the DNA fragments are exposed to a critical level of denaturant, the DNA strands begin to dissociate. This dissociation causes a significant reduction in the mobility of the fragment. The position in the gel at which the level of denaturant is critical for a particular DNA fragment is a function of the T<sub>m</sub> of the DNA fragment and is therefore different for wild-type versus mutant fragments. Consequently, upon migration to the position at which the level of denaturant is at the critical point, for either the wild-type or the mutant fragment, the mobility of these two molecules will become different, thus resulting in their separation. The mutation detection rate of DGGE approaches 100%. Although the technique of DGGE is relatively simple to perform, and does not require radioisotopes or toxic chemicals, it does require some specialized equipment. Furthermore,



DGGE can only be used to analyze fragments between 100 and 800bp due to the resolution limit of polyacrylamide gels. DGGE is advantageous over other methods useful for detecting sequence variations because the behavior of DNA molecules on DGGE gels can be modeled by computer thereby making it possible to accurately predict the detectability of a mutation in a given fragment.

- 5 Genomic DNA fragments can be efficiently transferred from the gel following DGGE as described in US Patent No. 5,190,856.

#### Chemical Cleavage of Mismatches

- 10 Chemical cleavage of mismatch (CCM) is another technique for detection of sequence variations that is useful according to the invention. CCM is based upon the ability of hydroxylamine and osmium tetroxide to react with the mismatch in a DNA heteroduplex and the ability of piperidine to cleave the heteroduplex at the point of mismatch. According to the method of CCM, sequence variations are detected by the appearance of fragments that are smaller than the untreated heteroduplex following denaturing polyacrylamide gel electrophoresis.

- 15 DNA fragments up to 1kb in size can be analyzed by CCM with a probable 100% detection rate for sequence variation. CCM is particularly useful for either detecting all of the sequence variations in a particular fragment of DNA or for determining that there are no sequence variations in a particular fragment of DNA.

#### 20 Constant Denaturant Capillary Electrophoresis (CDCE) Analysis

- CDCE analysis is particularly useful in high throughput screening, i.e., wherein large numbers of DNA samples are analyzed. CDCE analysis combines several elements of both replaceable linear polyacrylamide capillary electrophoresis and constant denaturant gel electrophoresis. The technique of CDCE is a rapid, high resolution procedure that demonstrates a high dynamic range, and is automatable. The method of CDCE, as described in detail in Khrapko et al., 1994, Nucleic Acids Res. 22:364, involves the use of a zone of constant temperature and a denaturant concentration in capillary electrophoresis. Linear polyacrylamide gel electrophoresis is performed at viscosity levels that permit facile replacement of the matrix after each run. For a typical 100 bp fragment of DNA, point mutation-containing heteroduplexes are separated from wild type homoduplexes in less than 30 minutes. Using laser-induced fluorescence to detect fluorescent-tagged DNA, the system has an absolute limit of detection of  $3 \times 10^4$  molecules with a linear dynamic range of six orders of magnitude. The relative limit of detection is about 3/10,000, i.e., 100,000 mutant sequences are recognized among  $3 \times 10^8$  wild type sequences. This approach is applicable to analysis of low frequency mutations, and

to genetic screening of pooled samples for detection of rare variants.

### Rnase Cleavage

An additional method for genotyping that is useful according to the invention is RNase  
5 Cleavage. Various ribonuclease enzymes, including RNase A, RNase T1 and RNase T2 specifically digest single stranded RNA. When RNA is annealed to form double stranded RNA or an RNA/DNA duplex, it can no longer be digested with these enzymes. However, when a mismatch is present in the double stranded molecule, cleavage at the point of mismatch may occur.

RNase Cleavage is preferably performed with RNase A. Ribonuclease A specifically digests  
10 single stranded RNA but can also cleave heteroduplex molecules at the point of mismatch. The extent of cleavage at single base mismatches depends on both the type of mismatch, and the sequence of DNA flanking the mismatch. Sequence variations leading to mismatch are indicated by the presence of fragments that are smaller than the uncleaved heteroduplex on denaturing polyacrylamide gels.

According to the invention, RNase Cleavage involves forming a heteroduplex between a  
15 radiolabeled single stranded RNA probe (riboprobe) and a PCR product derived from a biological sample. If a point mutation is present in the PCR product, following treatment of the resulting RNA/DNA heteroduplex with RNase A, the RNA strand of the duplex may be cleaved. The sample is then denatured by heating and analyzed on a denaturing polyacrylamide gel. If the RNA probe has not been cleaved, it will be the same size as the PCR product. If the probe has been cleaved, it will be  
20 smaller than the PCR product. RNase Cleavage can be used to easily detect a 1 bp deletion. However, small insertions may not be as easily detected as small deletions, by RNase Cleavage, as 'looping-out' occurs on the target strand rather than the probe strand.

### Heteroduplex Analysis

25 Another method for genotyping according to the invention is heteroduplex analysis. Heteroduplex molecules, i.e., double stranded DNA molecules containing a mismatch, can be separated from homoduplex molecules on ordinary gels. The exact rate of detection of sequence variations by heteroduplex analysis is unknown, but is clearly significantly lower than 100%. Presumably, the sequence of DNA flanking the mismatch, rather than the actual mismatch affects the  
30 detectability. Mismatches that are located in the middle of a DNA fragment are detected most easily. Although heteroduplex analysis is less sensitive than some of the other genotyping methods described, it may be considered useful according to the invention due to its simplicity.

### Mismatch Repair Detection (MRD)

Another technique that is useful for genotyping according to the invention is mismatch repair detection (MRD). MRD is an *in vivo* method that detects DNA sequence variation by the occurrence of a change in bacterial colony color. DNA fragments to be screened for variation are cloned into two MRD plasmids, and bacteria are transformed with heteroduplexes of these constructs. The resulting colonies are blue in the absence of a mismatch and white in the presence of a mismatch. MRD can be used to detect a single mismatch in a DNA fragment as large as 10 kb in size. MRD permits high-throughput screening of genetic mutations, and is described in detail in Faham et al., 1995, *Genome Research* 5:474.

### Mismatch Recognition by DNA Repair Enzymes

Another technique that is useful for detecting sequence variations according to the invention is Mismatch Recognition by DNA Repair Enzymes. The E.coli mismatch correction systems are well-understood. Three of the proteins required for the methyl-directed DNA repair pathway: MutS, MutL and MutH are sufficient to recognize 7 of the possible 8 single base-pair mismatches (C/C mismatches are not recognized) and cut/nick the DNA at the nearest GATC sequence. The MutY protein, which is involved in a distinct repair system can also be used to detect A/G and A/C mismatches. Some mammalian enzymes are also useful for mismatch recognition: thymidine glycosylase can recognize all types of T mismatch and 'all-type endonuclease' or Topoisomerase I is capable of detecting all 8 mismatches, but does so with varying efficiencies, depending on both the type of mismatch and the neighboring sequence.

The MutS gene product is the methyl-directed repair protein which binds to the mismatch. Purified MutS protein has been used to detect mutations by several different methods. Gel mobility assays can be performed in which DNA bound to the MutS protein migrates more slowly through an acrylamide gel than free DNA. This method has been used to detect single base mismatches.

An alternative method for the use of MutS in mismatch recognition, which does not require gel electrophoresis, involves the immobilization of MutS protein on nitrocellulose membranes. Labeled heteroduplexed DNA is used to probe the membrane in a dot-blot format. When both DNA strands are used, all mismatches can be recognized by binding of the DNA to the protein attached to the membrane. Although C/C mismatches are not detected, the corresponding G/G mismatch derived from the other strand is recognized. This technique is particularly useful because it is simple, inexpensive, and amenable to automation. However, the detection efficiency of this method may be limited by the size of the DNA fragment. In particular, this method works well for very short

fragments.

### Sequencing by Hybridization (SBH)

An alternative method for detecting sequence variations according to the invention is sequencing by hybridization (SBH). According to this method, arrays of short (8-10 base long) oligonucleotides are immobilized on a solid support in a manner similar to the reverse dot-blot protocol, and probed with a target DNA fragment. In particular, oligonucleotides are synthesized together and directly onto the support.

The synthesis system begins with a silicon chip coated with a nucleotide linked to a light-sensitive chemical group which is used to illuminate particular grid co-ordinates removing the blocking group at these positions. The chip is then exposed to the next photoprotected nucleotide, which polymerizes onto the exposed nucleotides.

In this manner, as a result of successive rounds of nucleotide additions, oligonucleotides of different sequences can be synthesized at different positions on the solid support. Thirty-two cycles of specific additions (i.e., 8 additions of each of the four nucleotides) should enable the production of all 65,536 possible 8-mer oligonucleotides at defined positions on the chip.

When the chip is probed with a DNA molecule, e.g., a fluorescently labeled PCR product, fully matched hybrids should give a high intensity of fluorescence and hybrids with one or more mismatches should give substantially less intense fluorescence. The combination of the position and intensity of the signals on the chip enables computers to derive the sequence of the DNA molecule being analyzed for the presence of sequence variations.

### Allele-Specific Oligonucleotide Hybridization

The technique of allele-specific oligonucleotide (ASO) hybridization or the 'dot-blot' is also useful for genotyping according to the invention. Under specific hybridization conditions, an oligonucleotide will only bind to a PCR product if the two are 100% identical. A single base pair mismatch is sufficient to prevent hybridization. A pair of oligonucleotides, one carrying the wild type base and the other carrying a single base change, as compared to the wild type sequence, can be used to determine if a PCR product is homozygous wild type, heterozygous or homozygous mutant for a particular base change. When performing conventional dot blots, the PCR product is fixed onto a nylon membrane and probed with a labeled oligonucleotide. When performing a 'reverse dot blot', an oligonucleotide is fixed to a membrane and probed with a labeled PCR product. The probe may be isotopically labeled, or non-isotopically labeled. The technique allows for the genotyping of multiple

PCR amplified samples for the presence of a single base change.

### Allele-Specific PCR

Many methods for identifying sequence variations involve the analysis of PCR-amplified DNA. The allele-specific polymerase chain reaction (also called the amplification refractory mutation system or ARMS) comprises an assay that occurs during the PCR reaction itself. ARMS requires the use of sequence-specific PCR primers which differ from each other at their terminal 3' nucleotide and are designed to amplify only the normal allele in one reaction, and only the mutant allele in another reaction. When the 3' end of a specific primer is 100% identical to the target, amplification occurs. When the 3' end of a specific primer is not 100% identical to the target, amplification does not occur. Agarose gel electrophoresis is used to detect the presence of an amplified product. The genotype of a (heterozygous) wild-type sample is characterized by amplification products in both reactions, and a homozygous mutant sample generates product in only the mutant reaction.

This technique can be modified so that the 5' ends of the allele-specific primers are labeled with different fluorescent labels, and the 5' end of the common primers are biotin labeled. According to this alternate protocol, the wild-type specific and the mutant-specific reactions are performed in a single tube. The advantages of this approach are that a gel electrophoresis step is not required, and the method is amenable to automation.

### Primer-Introduced Restriction Analysis

The method of primer-introduced restriction analysis (PIRA) can also be used for genotyping according to the invention. PIRA is a technique which allows known sequence variations to be detected by restriction digestion. By introducing a base change close to the position of a known sequence variation (for example by using a PCR primer containing a mismatch, as compared to the target sequence), it is possible to create a restriction endonuclease recognition site that indicates the presence of a particular sequence change. The combination of the altered base in the primer sequence and the altered base at the mutation site, creates a new restriction enzyme target site. This approach may be used to create a new restriction enzyme site in either the wild-type allele or the mutant allele. If a novel restriction enzyme site is introduced in the mutant allele then, following digestion with the appropriate restriction enzyme, the homozygous wild-type form would produce a single band of the full-length size, the homozygous mutant form would produce a single band of the reduced size and the heterozygous form would produce both full length and reduced sized bands. Band size will be analyzed by gel electrophoresis.

### Oligonucleotide Ligation Assay

The technique of oligonucleotide ligation can also be used for genotyping according to the invention.

The method of oligonucleotide ligation is based on the following observations. If two  
5 oligonucleotides are annealed to a strand of DNA and are exactly juxtaposed, they can be joined by the enzyme DNA ligase. If there is a single base pair mismatch at the junction of the two oligonucleotides then ligation will not occur. According to the method of oligonucleotide ligation, the two oligonucleotides used in the assay are modified by the addition of two different labels. According to this method, the assay for a ligated product involves detecting a ligated product by assaying for the  
10 appearance of the labels of the two oligonucleotides on a single molecule rather than visualization of a new, larger sized DNA fragment by gel electrophoresis.

When ligation reactions are conducted in 96-well microtiter plates and ligation is scored by ELISA, the oligonucleotide ligation assay can be performed by a robot and the results can be analyzed by a plate reader and fed directly into a computer. This method is therefore extremely useful for  
15 detecting the presence of a sequence variation in a large number of samples. The oligonucleotide ligation assay is performed on PCR-amplified DNA. A modification of this assay, termed the ligase chain reaction, is performed on genomic DNA and involves amplification with a thermostable DNA ligase.

### 20 Direct DNA Sequencing

Genotyping according to the invention may also be carried out by directly sequencing the DNA sample in the region of the gene of interest, using DNA sequencing procedures well-known in the art (described above in Section D, entitled "Isolation of a Wild Type Gene").

### 25 Mini-Sequencing

The technique of mini-sequencing (also known as single nucleotide primer extension) can also be used to detect any known point mutation, deletion or insertion, according to the invention. Obtaining sequence information for just a single base pair only requires the sequencing of that particular base. This can be done by including only one base in the sequencing reaction rather than all four. When this  
30 base is labeled and complementary to the first base immediately 3' to the primer (on the target strand), the label will not be incorporated. Thus, a given base pair can be sequenced on the basis of label incorporation or failure of incorporation without the need for electrophoretic size separation.

### 5' Nuclease Assay

Genotyping according to the invention can also be performed by the method of 5' nuclease assay. The 5' nuclease assay is a technique that monitors the extent of amplification in a PCR reaction on the basis of the degree of fluorescence in the reaction mix. A low level of fluorescence indicates no amplification or very poor amplification and a high level of fluorescence indicates good amplification. This system can be adapted to permit identification of known sequence variations, without the need for any post-PCR analysis other than fluorescence emission analysis.

PCR amplification is detected by measuring the 5' to 3' exonuclease activity of Taq polymerase. Taq polymerase cleaves 5' terminal nucleotides of double stranded DNA. The preferred substrate for Taq polymerase is a partially double stranded molecule. Taq polymerase cleaves the strand that contains the closest free 5' end. According to the 5' nuclease assay, an oligonucleotide 'probe' which is phosphorylated at its 3' end so as to render it incapable of serving as a DNA synthesis primer, is included in the PCR reaction. The probe is designed to anneal to a position between the two amplification primers. When an actively extending Taq polymerase molecule reaches the probe molecule, it partially displaces the probe and then cleaves the probe at or near the single stranded/double stranded cleavage site until the entire probe is broken up and removed from the template. The polymerase continues this process of displacement and cleavage until the entire probe is broken up and removed from the template. The probe is labeled in a manner that permits detection of the removal of the probe. In particular, the probe is labeled at different positions with two different fluorescent labels. One label has a localized quenching effect on the fluorescence of the other (reporter) label. This effect is mediated by energy transfer from one dye to the other, and requires that the two dyes are in close proximity to each other. If the probe is cleaved at a position between the reporter and the quencher dyes, the two dyes become physically separated thereby resulting in an increase in fluorescence which is proportional to the yield of the PCR product.

### Representational Difference Analysis (RDA)

Genotyping according to the invention can also be carried out by Representational Difference Analysis (RDA). RDA is described in detail in Lisitsyn et al., 1993, Science 259:946, and an adaptation which combines selective breeding with RDA is described in Lisitsyn et al., 1993, Nature Genet. 6:57. RDA identifies sequence dissimilarities through the application of a powerful approach to subtractive hybridization. According to the method of RDA, one first creates simplified representations, called amplicons, from two samples that are being compared. An amplicon can comprise, for example, the set of BglIII fragments that are small enough to be amplified by the PCR.

The iterative subtraction step begins with the ligation of a special adaptor to the 5' end of fragments contained in the amplicon derived from the test sample (tester amplicon). The tester amplicon is then melted and briefly reannealed in the presence of a large excess of amplicon, derived from the wild type sample (driver amplicon). Those tester fragments that reanneal (presumably fragments absent from the wild type, driver amplicon) can serve as a template for the addition of the adaptor sequence to the 3'-end of the "partner" fragment. As a result, these tester fragments can be exponentially amplified by PCR. This procedure is then repeated to achieve successively higher enrichment.

RDA may be used to clone sequences that are either wholly absent from the wild type sample or are present in the wild type DNA, but are contained in a restriction fragment that is too large to be amplified in the amplicon. The former case may arise from a total deletion; the latter from a restriction fragment length polymorphism with the short allele present in the tester but not the wild type DNA. RDA is useful for subtracting DNA from an individual with a particular disease from normal DNA so as to identify regions showing homozygous or heterozygous deletions; locating fragments present in a parent with a dominant disorder but absent in his unaffected offspring; and locating mRNAs expressed in normal tissue but not present in tissue isolated from an individual with a particular disease.

#### Denaturing High Performance Liquid Chromatography

According to the scanning method of Denaturing High Performance Liquid Chromatography (DHPLC), partial heat denaturation and a linear acetonitrile column are used to identify polymorphisms in DNA fragments. DHPLC provides a method of comparative DNA sequencing based on the capability of ion-pair reverse phase liquid chromatography on alkylated nonporous poly(styrene divinylbenzene) particles to resolve homo- from heteroduplex molecules under conditions of partial denaturation. This method can potentially be automated to allow for rapid analysis of a large number of samples (Underhill et al., 1996, Proc. Natl. Acad. Sci. USA, 93:196).

#### Mass Spectroscopy

Matrix-assisted laser desorption-ionization-time-of-flight (MALDI-TOF) mass spectroscopy is another method according to the invention by which genotyping can be performed. The method of MALDI-TOF mass spectroscopy is based on the irradiation of crystals formed by suitable small organic molecules (referred to as the matrix) with a short laser pulse at a wavelength close to the resonant adsorption band of the matrix molecules. This causes an energy transfer and desorption process producing matrix ions. Low concentrations of nucleic acid molecules are added to the matrix molecules while in solution and become embedded in the solid matrix crystals upon drying of the



mixture. The intact nucleic acids are then desorbed into the gas phase and ionized upon irradiation with a laser allowing their mass analysis. MALDI is used primarily with time-of-flight spectrometers where the time of flight is related to the mass-to-charge ratio of the nucleic acids molecules. Reviewed in Griffin T.J. and Smith L.M., 2000, Trends Biotech 18:77.

5 Genotyping can be performed by any of the following MALDI-TOF mass spectroscopy approaches including sequencing of PCR products (Fu, D-J et al., 1998, Nat. Biotechnol. 16:381; Kirpekar, F. et al., Nucleic Acids Res. 26:2554), direct mass-analysis of PCR products (Ross, P.L. et al., 1998, Anal. Chem. 70:2067), analysis of allele-specific PCR (Taranenko, N.I. et al, 1996, Genet. Anal. Biomol. Eng. 13:87) or LCR (ligase chain reaction; Jurinke, C. et al., 1996, Anal. Biochem. 10 237:174) products, analysis of RFLP-PCR products (Srinivasan, J.R. et al., 1998, Rapid Commun. Mass Spectrom. 12:1045), minisequencing (Haff, L.A. and Smirnov, I.P., 1997, Genome Res. 7:378; Higgins, G.S. et al., 1997, BioTechniques 23:710), analysis of PNA (peptide nucleic acid) hybridization probes (Griffin, T.J. et al., 1997, Nat. Biotech. 15:1368; Ross, P.L., Anal. Chem. 69:4197; Jiang-Baucom, P. et al., 1997, Anal. Chem. 69:4894), or direct analysis of invasive cleavage products 15 (Griffin, T.J. et al, 1999, Proc. Natl. Acad. Sci. USA 96:6301).

## 6. Methods of Specifying a Polymorphism

The invention provides methods for specifying a particular polymorphism. By "specifying a polymorphism" is meant defining a polymorphism in the context of a larger region of nucleic acid which contains the polymorphism, and is of sufficient length to be easily differentiated from any other position in the genome.

A unique nucleotide position (e.g. a polymorphic site) in the human genome can be specified by describing a unique sequence of DNA within the genome, and providing the location of the unique nucleotide position relative to that sequence. Preferably this is done by providing the sequence identity 25 of a length of unique DNA containing the polymorphism, and indicating which of the nucleotide sites is polymorphic.

A calculation can be made to determine a sequence length which will be unique in the 3 billion nucleotide human genome. If it is assumed that the genome contains equal numbers of the nucleotides A, G, C and T, and that they occur randomly in the genome, one can determine the probability of any 30 given sequence of a defined length occurring in the genome; a random 12mer will appear in a random 3,000,000,000 bp genome 179 times, a random 15 mer will appear in a random 3,000,000,000 bp genome 3 times and a random 16mer will appear in a random 3,000,000,000 bp genome 1 time.

Thus, it would appear that specifying 16 bp would uniquely define a sequence in the genome.

However, the genome is not composed of random sequence and does not contain equal amounts of A, G, C and T. In fact, 10-12 bp sequences are likely to be specific for 95% of genes. Some sequences may even be specified by as few as 8 nucleotides. The minimum sequence length that is useful according to the invention for identifying polymorphisms in most gene and intergenic sequences is approximately 9-15 bp.

In the case of repeat sequences and sequences associated with gene families, the probability of observing a particular sequence is greatly increased and it becomes difficult to specify a polymorphism in the context of a sequence that is only on the order of 9-15 bp. There are many types of repeats including tandem repeats, where a larger sequence block has within it smaller repeat units (e.g. microsatellites). Tandem repeats usually occur within non-genic areas, but can also occur within genes and subsequently affect gene function; they can be 10-1000s of bp long, or, if located in centromeres and telomeres, be megabase sized. Some repeats are composed of blocks which do not have sub-repeat units and are non-functional (e.g. ~300 bp Alu repeats). These occur by duplication/dispersal throughout the genome.

It may be difficult to specify a polymorphism that occurs in a gene that is a member of a gene family. Through the mechanism of gene duplication, gene families, comprising multiple copies of a gene in which some, but not all of the DNA sequence has diverged, have been formed. Thus, certain regions of a gene may be conserved in different gene family members. With time, a duplicated gene can lose function and the sequence of the duplicated gene can deteriorate; the amount of homology between the original gene and the duplicated version depends upon the time since duplication. Other duplications maintain function and retain some level of similarity with the original gene in the important domains. Some related genes can share nearly 100% homology across a region that is hundreds of bp long, and yet have no significant homology at any other location. In these cases, it may be necessary to specify dozens or more nucleotides to provide a unique sequence.

To identify a unique sequence, a search must be done wherein a specific sequence is compared to all known human sequences and the minimum unique sequence is defined. However, in the absence of a complete sequence for the human genome, it cannot be guaranteed that a sequence is truly unique. Empirical experimentation can be used to determine the minimum sequence for specificity/uniqueness. In the case of a gene family member, if sequence information is available for the region corresponding to the region of interest in other members of the gene family, then it may be possible to define a unique short (9-15 bp) sequence that contains a polymorphism and has specificity. In the event that a particular region cannot be defined as unique, a larger region of nucleic acid which contains the polymorphism will be required to define a polymorphism in a gene that is a member of a

gene family. It is predicted that a sequence of 9-15 bp will be sufficient to define a polymorphism in 99% of all cases.

Methods of specifying a polymorphism that involve using sequences which either encompass or overlap the polymorphic site to be tested or do not encompass or overlap the polymorphic site to be tested are useful according to the invention and are described below.

#### Oligonucleotide Hybridization.

An oligonucleotide is designed such that it is specific for a target sequence, and hybridizes only at the target sequence site. This oligonucleotide will not hybridize if the target sequence differs at the position in the sequence to be tested. Another oligonucleotide is designed such that it hybridizes with the polymorphic form of the sequence. A DNA sample is tested for hybridization with each of the two probes independently. If the DNA hybridizes to only one of the probes, it can be concluded that the individual is homozygous for the corresponding sequence. If both probes hybridize to a test DNA sample, then the individual is heterozygous. Hybridization will be detected by the method of Southern blot analysis (as described in Section C entitled "Production of a Nucleic Acid Probe").

#### Specifying a Polymorphism by PCR

An alternative method for specifying a particular polymorphism involves a PCR-based strategy. According to this method, a region of a candidate gene to be tested is amplified by PCR (as described). The amplified fragment is digested with a restriction enzyme that will not cut a fragment that contains a polymorphism, due to the location of the polymorphism within the recognition site of this restriction enzyme. The products of the digestion reaction mixture are size separated in an agarose gel, stained with ethidium bromide, and visualized under ultraviolet light to determine if the amplified product has been digested. According to this method, the PCR primers provide the specificity for a particular polymorphism by virtue of the specific sequence of the two primers, as well as by the location of the primer binding sites in the target DNA. Although, multiple sites for primer binding may exist in a target DNA sequence, only the sites that are close enough together will produce an amplified product that includes the nucleic acid region containing the polymorphism.

Alternatively, a PCR reaction is carried out with PCR primers that contain polymorphisms. According to this embodiment, if the template nucleic acid lacks the polymorphism present in the primers there will be no PCR product. Thus, according to this embodiment of the invention, the absence of a PCR product indicates that a polymorphism is not present in the target sequence.

### Primer Extension

A DNA fragment comprising the region containing a polymorphism is PCR amplified from an individual to be tested. The PCR product is denatured and one strand is retained for analysis. An oligonucleotide probe is designed such that it is specific for a region in the sequence and hybridizes such that its 3' terminal nucleotide is paired with the nucleotide adjacent to the one to be tested. The PCR product and probe are combined with a polymerase and terminating, differentially colored, nucleotides. The polymerase extends the probe by one base, and only the base which is complementary to the site being tested is added. The reaction is washed, and the color of the reaction indicates the nucleotide that has been added and the sequence at the position of interest.

The PCR step provides one level of specificity by amplifying a region (1 - 10000 bp as desired between the PCR primers) from a complex (3,000,000,000 bp) mixture. The PCR probes primers must be unique in both their hybridization specificity and their proximity to one another. Since proximity of the two PCR primers is needed (i.e. a distance across which a polymerase can extend to join the primers), shorter PCR primers can be used, e.g. in theory a small enough region could be amplified with a 8-10 bp binding site for a PCR primer. To ensure that a primer hybridizes with specificity, a primer must be at least 5 bp.

A second level of specificity is provided by the primer which is extended in the primer extension reaction. Since this primer is hybridizing to a short piece of DNA, it can be short and unique for the fragment with which it binds. The primer is at least 5bp and preferably 8bp. Although the primer used for the primer extension step is located probe adjacent to the polymorphic site, the PCR primers should not overlap with the polymorphic site being tested.

### Southern Blotting

One method for detecting a previously defined polymorphism involves Southern blot analysis of wild type and mutant DNA following digestion with a restriction enzyme which has a recognition sequence which includes the polymorphic site to be tested. According to this method, a particular restriction enzyme cuts wild type DNA but does not cut mutant DNA due to the presence of a polymorphism within the recognition site of this restriction enzyme. Many restriction enzymes exist which recognize 4bps. The resulting fragments will be size separated in an agarose gel, transferred to a membrane and probed with a nucleic acid probe. If the site is uncut, the fragment is one length and if the site is cut the fragment will be of a shorter length.

The nucleic acid hybridization probe will provide specificity to the particular polymorphism being tested by defining the polymorphism in the context of a larger stretch of nucleic acid sequence.

The nucleic acid probe may comprise the nucleic acid sequence corresponding to the region known to contain the polymorphism. The sequence-specific probe may be located 10, 100, 1000, or even 100s of thousands of bases from the region containing the polymorphism. If the probe is located some distance from the region containing the polymorphism, an intervening recognition site for the restriction enzyme cannot be located between the probe hybridization site and the region of interest containing the polymorphism site. Typically, a hybridization probe useful according to this method will be much larger than the minimum length of a sequence (9-15 bp) required to give specificity to, or define a particular polymorphism.

Alternatively, a chemical or enzyme which recognizes a unique pair of nucleotides at the site of a polymorphism, can be used to detect the polymorphism. According to this method, the amount of sequence required for recognition by a chemical or enzyme is 2 bp (providing that the 2 bp sequence is unique in a region large enough to produce a fragment which can then be bound by a specific probe).

According to a variation of the above method, a labeled chemical or enzyme which binds to one sequence of the polymorphic recognition site and not another is used. This method involves the steps of digesting the DNA with a restriction enzyme, and adding a labeled, sequence-specific binding protein (e.g. a restriction enzyme that lacks cleavage capability). The sequence-specific binding protein will bind to multiple sites in the genome, including the site to be tested. The fragments will be separated on a gel and then probed with a probe specific for the test sequence. If the fragment identified by the second probe is identical to a fragment identified by the first probe (e.g. the labeled chemical or enzyme), then the sequence being tested for is present.

## 7. Determination of the Phenotypic Outcome of a Polymorphism

To determine the phenotypic outcome of a polymorphism according to the invention, it is necessary to screen suitable populations to obtain a statistically significant measure of the association of a polymorphism with a particular disease (e.g. osteoarthritis). The invention provides methods for performing polymorphism genotyping in appropriate populations (described above). The invention also provides *in vitro* and *in vivo* assays useful for determining the phenotypic outcome of a polymorphism in a candidate gene.

Every polymorphism has the potential to alter the genetic activity of an individual. At the level of a single gene, the effect of a polymorphism can range from an inconsequential, silent change to a change that causes a complete loss of protein function to a gain of aberrant or detrimental function mutation. The severity of the effect of a polymorphism on gene activity will depend on the exact

molecular consequences of the particular polymorphism. For example, alterations of a single pre-mRNA splicing dinucleotide could have profound effects on both the quantitative and qualitative properties of gene activity since alterations in splicing efficiency can both reduce the overall level of normal transcription as well as cause "exon skipping". If the deleted exon involves a coding exon then exon skipping will lead to an alteration in the amino acid composition of the resulting protein and likely effect protein activity. To accurately assess the role of a particular polymorphism in the regulation of various molecular events, appropriate assays for both gene expression and protein function must be carried out.

*In vitro* assays useful for determining the effects of a polymorphism on gene expression and protein function include, but are not limited to the following.

i. Transcriptional Regulation

The transcriptional regulation of a candidate gene containing a polymorphism may be altered, as compared to the wild type gene.

Promoter Activity

If a polymorphism is located in the promoter, enhancer or repressor region of a candidate gene, promoter assays (well known in the art) wherein the altered promoter of the candidate gene is used to drive the expression of a reporter gene (e.g. CAT, luciferase, GFP) are performed. Changes in the transcriptional regulation of a candidate gene due to the presence of a polymorphism can also be detected by methods useful for measuring the level of mRNA including S1 nuclease mapping and RT-PCR.

S1 Analysis

The S1 enzyme is a single-stranded endonuclease that will digest both single-stranded RNA and DNA. According to the method of S1 analysis, a probe that has been efficiently labeled to a high specific activity at the 5' end through the use of a kinase, is used to determine either the amount of an mRNA species or the 5' end of a message. A single stranded probe that is complementary to the sequence of the RNA species of interest is utilized in S1 analysis. If the structure of a particular mRNA species is known, S1 analysis is performed with oligonucleotide probes of at least 40 bp, that are complementary to the RNA of interest. It is preferable to use oligonucleotides wherein the 5' end of the oligonucleotide is complementary to the RNA. It is also preferable to use oligonucleotides wherein the 5' terminal residues contain dG or dC residues. If S1 nuclease analysis will be utilized to determine the 5' termini of an RNA species, the 3' end of the oligonucleotide should extend at least 4

nucleotides beyond the RNA coding sequence. The inclusion of additional nucleotides facilitates differentiation of a band resulting from an RNA:DNA duplex and a band representing the probe.

A hybridization probe for S1 analysis is prepared by incubating 2pmol of an oligonucleotide in the presence of 150 mCi [ $^{32}$ P]ATP (3000-7000Ci/mmol), 2.5 ml 10X T4 polynucleotide kinase buffer (700mM Tris-Cl, pH 7.5, 100 mM MgCl<sub>2</sub>, 50 mM dithiothreitol, 1 mM spermidine-Cl, 1 mM EDTA),  
 5 and 10U T4 polynucleotide kinase for 37°C for 30-60 minutes. The radiolabeled probe is ethanol precipitated and resuspended at 1ml/0.3ng oligonucleotide or 10<sup>5</sup> cpm.

The hybridization reaction is performed as follows. An amount of probe equal to 5x10<sup>4</sup> Cerenkov counts is added to 50mg RNA on ice and ethanol precipitated. The resulting pellet is  
 10 resuspended in 20ml S1 hybridization solution (80% deionized formamide, 40 mM PIPES, pH 6.4, 400mM NaCl, 1 mM EDTA, pH 8), denatured for 10 min at 65°C and hybridized overnight at 30°C. The following day, 300 ml of a mixture of 150 ml 2x S1 nuclease buffer (0.56M NaCl, 0.1 M sodium acetate, pH 4.5, 9mM ZnSO<sub>4</sub>), 3ml 2mg/ml single-stranded calf thymus DNA, 147 ml H<sub>2</sub>O and 300U S1 nuclease is added to the hybridization reaction and incubated for 60 minutes at 30°C. Following the  
 15 addition of 80ml S1 stop buffer (4M ammonium acetate, 20mM EDTA, 40 mg/ml tRNA) the sample is ethanol precipitated, resuspended in formamide loading dye, denatured and analyzed on a denaturing polyacrylamide/urea gel of the appropriate percentage for the expected size of the protected band (Ausubel et al., supra).

## 20 RT-PCR

The method of RT-PCR is useful according to the invention for RNA expression analysis. According to the method of reverse transcription /polymerase chain reaction (RT-PCR) during the reverse transcription (RT) step, the RNA is converted to first strand cDNA, which is relatively stable and is a suitable template for a PCR reaction. In the second step, the cDNA template of interest is  
 25 amplified using PCR. This is accomplished by repeated rounds of annealing sequence- specific primers to either strand of the template and synthesizing new strands of complementary DNA from them using a thermostable DNA polymerase.

An RNA sample is ethanol precipitated with a cDNA primer. It may be preferable to use a cDNA primer that is identical to one of the amplification primers. To the pellet is added 12 ml H<sub>2</sub>O,  
 30 4ml 400mM TrisCl, pH 8.3, and 4 ml 400 mM KCl. The mixture is heated to 90°C, slow cooled to 67°C, microfuged and incubated for 3 hours at 52°C. Following the addition of 29ml reverse transcriptase buffer (per sample/2.5ml 400mM TrisCl, pH8.3, 2.5ml 400mM KCl, 1ml 300mM MgCl<sub>2</sub>, 5ml 100mM DTT, 5ml 5mM 4 dNTP mix, 2ml actinomycin D, 11ml H<sub>2</sub>O) and 0.5ml (16U) AMV

reverse transcriptase, the sample is incubated for 1 hour at a temperature between 37°C and 55°C.

The temperature will be adjusted in accordance with the composition of the primer and the RNA of interest. The sample is then extracted sequentially with phenol and chloroform, and ethanol

precipitated. The resulting cDNA pellet is resuspended in 40ml H<sub>2</sub>O. 5ml of the cDNA sample is

5 mixed with 5ml of each amplification primer (~20mM each), 4ml 5mM 4dNTP mix, 10ml 10X amplification buffer (500mM KCl, 100mM TrisCl, pH8.4, 1mg/ml gelatin) and 70.5ml H<sub>2</sub>O. After the

mixture is heated for 2 minutes at 94°C, 0.5 ml (2.5U) Taq DNA polymerase is added and the sample

is overlaid with mineral oil. PCR amplification of the cDNA will be performed using the following

automated amplification cycles: 39 cycles (2 minutes at 55°C, 2 minutes at 72°C, 1 minute at 94°C), 1

10 cycle (2 minutes at 55°C, 7 minutes at 72°C). The number of cycles can be varied in accordance with

the abundance of RNA (Ausubel et al., supra).

If a polymorphism is located in a transcription factor binding site, assays including but not limited to the yeast two-hybrid assay (Fields et al., 1994, Trends Genet., 10:286) can be used to determine the effects of a polymorphism on transcription factor binding.

15 If the protein product of the gene of interest is a DNA binding protein the phenotypic outcome of a polymorphism may be impaired nuclear transport, DNA binding, chromatin assembly or chromatin structure, methylation or histone deacetylation.

### Nuclear Transport

20 Immunocytochemical methods or cell fractionation techniques (as described above) are used to determine if the protein is correctly localized in the nucleus.

The DNA binding properties of a transcription factor are determined by gel shift analysis (as described in Ausubel et al., supra), oligonucleotide selection, southwestern assays or by immunohistochemical analysis of fixed chromosomes.

### Gel Shift Analysis

25 The method of gel shift analysis is used to detect sequence specific DNA-binding proteins from crude extracts. According to this method, proteins that bind to an end-labeled DNA fragment will retard the mobility of the fragment. The change in the mobility of the labeled fragment is detected by the appearance of a discrete band comprising the DNA-protein complex.

30 A number of methods for preparing nuclear and cytoplasmic extracts useful for gel shift analysis are known in the art. For example, nuclear extracts are prepared according to the following method. A cell pellet is washed in PBS, resuspended in a volume of hypotonic buffer (10 mM HEPES,



pH 7.9, 1.5 mM MgCl<sub>2</sub>, 10mM KCl, 0.2 mM PMSF, 0.5 mM DTT ) that is approximately equal to 3 times the packed cell volume and allowed to swell on ice for 10 minutes. Cells are homogenized in a glass Dounce homogenizer and the nuclei are collected by centrifugation and resuspended in a volume of low-salt buffer (20 mM HEPES, pH 7.9, 25% (v/v) glycerol, 1.5 mM MgCl<sub>2</sub>, 0.02 M KCl, 0.2 mM EDTA, 0.2 mM PMSF, 0.5 mM DTT) equivalent to one-half of the packed nuclear volume. Following the addition of a volume of high-salt buffer (20 mM HEPES, pH 7.9, 25% (v/v) glycerol, 1.5 mM MgCl<sub>2</sub>, 1.2 M KCl, 0.2 mM EDTA, 0.2 mM PMSF, 0.5 mM DTT) equivalent to one-half of the packed nuclear volume (dropwise with stirring) to the nuclei, nuclear extraction is carried out for 30 minutes with continuous gentle stirring. The nuclei are collected by centrifugation and the nuclear extract is dialyzed against 50 volumes of dialysis buffer (20 mM HEPES, pH 7.9, 20% (v/v) glycerol, 100mM KCl, 0.2 mM EDTA, 0.2 mM PMSF, 0.5 mM DTT) until the conductivities of extract and buffer are equivalent. The extract is removed from the dialysis tubing and analyzed for protein concentration (Ausubel et al., supra).

Probes useful for gel shift analysis include a fragment of plasmid DNA or a gel-purified double stranded oligonucleotide. Preferably the probe is labeled with Klenow fragment by incubating a 100ml solution of plasmid DNA or oligonucleotide with 100mCi of the desired [ $\alpha$ -<sup>32</sup>P] dNTP, 4ml of 5 mM 3dNTP mix and 2.5 U Klenow fragment for 20 minutes at room temperature. Upon the addition of 4ml of a solution comprising 5 mM of the dNTP corresponding to the radioactive dNTP, the sample is incubated for 5 minutes at room temperature. The radiolabeled probe is ethanol precipitated, resuspended in TE buffer and gel purified.

Gel shift analysis is performed by incubating 10,000 cpm of the labeled probe (0.1-0.5 ng) with 2mg poly (dI-dC)-poly(dI-dC), 300 mg BSA, and approximately 15mg of a nuclear extract or buffered crude protein extract prepared, for example, as described above, for 15 minutes at 30°C. An aliquot of the binding reaction is analyzed by electrophoresis on a prewarmed low-ionic strength gel (e.g. a 4% polyacrylamide gel in TBE) and autoradiography (Ausubel et al., supra).

#### Oligoselection Assays for DNA Binding Activity

DNA binding activity is an essential property of proteins involved in many basic cell biological events, such as chromatin structure, transcriptional regulation, DNA replication and repair. The biological activity of a DNA binding protein can be assayed by defining the optimal target DNA binding site. Using the PCR based primer selection technique (Blackwell, 1990, Science, 250:1104) the canonical nucleotide sequence defining the binding site is elucidated *in vitro* by mixing purified full length protein, or just the DNA binding domain of a protein of interest, with an oligonucleotide duplex

pool containing a completely randomized central region flanked by primer-annealing sites. Multiple rounds of immunoprecipitation and amplification by PCR enriches for high affinity sites which are cloned and sequenced in order to define a canonical binding site.

The ability of a DNA binding protein to correctly regulate chromatin assembly and structure can be determined by DNase hypersensitivity assays. Alternatively, coimmunoprecipitation experiments or Western blot analysis can be used to determine if the DNA binding protein is associated with a component of the chromatin.

#### Southwestern Blot Assay for Protein-DNA Interactions

The ability of a protein to bind DNA is measured by using the "Southwestern" blot technique (for example see Antalis et al., 1993, Gene, 134:201). According to this method, radiolabelled DNA is incubated with protein that has been immobilized on nitrocellulose filters and the amount of bound DNA is measured by scintillation counting or autoradiography followed by densitometry. The protein to be tested can be pure protein, immunoprecipitated protein, crude cell lysates or even recombinant protein denatured directly from bacterial colonies, yeast or cell culture.

#### Assay of Protein Binding to Chromosomes *in Vivo*: Immunocytology of Fixed Chromosomes

Numerous biologically important nuclear proteins are in direct contact with genomic DNA. The presence of these proteins can be detected immunocytologically by fixing metaphase chromosomes such that the protein is permanently fixed at the region of DNA to which it normally binds. The presence and cytological location of the protein can then be determined by incubating the fixed chromosomes with an antibody directed against the protein of interest, and performing standard methods of immunohistochemical staining (Zink and Paro, 1989, Nature, 337:468).

#### Coimmunoprecipitation Assay for Chromatin Assembly/Structure

If an antibody specific for a protein of interest exists, immunoprecipitation can be used to test for the presence of the protein (Otto and Lee, 1993, Methods Cell Biol., 37:119, Banting, 1995, In Gene Probes 1: A practical approach. Chapter 8: Antibody probes, pp. 225-227, IRL press.). The following methods are used for determining if a protein of interest is associated with a particular subcellular component. According to one method, proteins are immunoprecipitated with an antibody specific for a cellular component (e.g. chromatin or nuclear antigens), the immunoprecipitated material is analyzed on a gel by denaturing polyacrylamide gel electrophoresis and western blot analysis is performed with an antibody specific for the protein of interest, to determine if a physical association

exists between the cellular component and the protein of interest. Various incubation and wash treatments of the cell lysate are used to remove background contamination and enhance the sensitivity of detection (Banting, 1995, supra). Alternatively, the initial immunoprecipitation can be carried out with the antibody specific for the protein of interest, and the western blot analysis can be performed  
5 with an antibody specific for a cellular component. According to a variation of this method, prior to immunoprecipitation the cells can be treated with a protein crosslinker to ensure that protein-protein interactions are maintained during immunoprecipitation. According to another variation of this method, proteins can be cross-linked to DNA and then precipitated (Dedon et al., 1991, Anal. Biochem., 197:83). If DNA coprecipitates with a particular protein, this suggests that DNA is associated with,  
10 and presumably bound to the protein. The coprecipitating DNA can be sequenced to identify the bound sequence.

#### DNAse Hypersensitivity

The transcriptionally active promoter region of a gene can be analyzed for susceptibility to  
15 cleavage by DNAseI (Montecino et al., 1994, Biochemistry, 33:348). Efficient cleavage of genomic DNA is dependent on the accessibility of this enzyme to the DNA, and is influenced by several factors, including nucleosome packaging, overall chromatin configuration, and the presence of DNA binding proteins such as transcription factors. DNA sequence variations within the promoter DNA may have profound effects on these factors and result in aberrant regulation of gene transcription and  
20 ultimately abnormal biological activity of the gene. Therefore, altered gene activity around a polymorphic site can be detected as increased or decreased DNAseI hypersensitivity (Vaishnaw et al., 1995, Immunogenetics, 41:354).

#### Assay for DNA Methylation

25 Accurate mapping of DNA methylation patterns, for example, in CpG islands which are unmethylated regions of DNA, is used to investigate and gain a better understanding of diverse biological processes such as the regulation of imprinted genes, X chromosome inactivation and tumor suppressor gene silencing in human cancer. DNA methylation at specific sites is most frequently studied by use of methylation-sensitive restriction endonucleases (for example HpaII) and Southern  
30 blotting (Sambrook et al., supra). The sensitivity of this method can be enhanced several hundred-fold by performing a ligation-mediated PCR step (as described in Steigerwald et al., 1990, Nucleic Acids Res., 6:1435) after enzyme treatment. An alternative strategy termed methylation-specific PCR (Herman et al., 1996, Proc Natl Acad Sci USA., 93:9821), is used to determine the methylation status

of CpG islands without the use of methylation-specific restriction enzymes.

### Histone-Deacetylation

Transcription of chromatin-packaged genes involves highly regulated changes in nucleosome  
5 structure that control DNA accessibility. Changes in nucleosome structure can be mediated by  
enzymatic complexes which control the acetylation and deacetylation of histones. Transcription  
elongation is required for the formation of the unfolded structure of transcribing nucleosomes, and  
histone acetylation is required for the maintenance of these structures (Walia et al., 1998, J. Biol.  
Chem., 3:14516). Deacetylation can be prevented by incubating cells with histone deacetylase  
10 inhibitors such as sodium butyrate or trichostatin A. To assay for changes in acetylation and the state  
of transcriptional activity, chromatin fractions are purified using organomercury and hydroxylapatite  
dissociation chromatographic techniques (Walia et al., supra).

#### ii. Transcription Start Site

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To determine if a particular polymorphism causes a change in the transcriptional start site of a  
candidate gene S1, nuclease mapping and primer extension can be performed. The presence of a  
polymorphism may cause an mRNA to be aberrantly expressed. In particular, a polymorphism may  
change the tissue specificity or developmental expression pattern of an mRNA species. A variety of  
20 molecular methods for detecting mRNA known in the art can be performed to determine the  
expression pattern of an mRNA. These methods include, but are not limited to the following: Northern  
blot analysis, RT-PCR, S1 analysis, RNase Protection analysis, or *in situ* hybridization analysis of  
sections, wherein the samples are derived from multiple different tissues or from a tissue at different  
stages of development. Northern blot analysis, RT-PCR and S1 analysis can also be used to determine  
25 if a polymorphism results in an altered pattern of mRNA splicing.

### Northern-Blotting

The method of Northern blotting is well known in the art. This technique involves the transfer  
of RNA from an electrophoresis gel to a membrane support to allow the detection of specific  
30 sequences in RNA preparations.

Northern blot analysis is performed according to the following method. An RNA sample  
(prepared by the addition of MOPS buffer, formaldehyde and formamide) is separated on an  
agarose/formaldehyde gel in 1X MOPS buffer. Following staining with ethidium bromide and

visualization under ultra violet light to determine the integrity of the RNA, the RNA is hydrolyzed by treatment with 0.05M NaOH/1.5M NaCl followed by incubation with 0.5M Tris-Cl (pH 7.4)/1.5M NaCl. The RNA is transferred to a commercially available nylon or nitrocellulose membrane (e.g. Hybond-N membrane, Amersham, Arlington Heights, IL) by methods well known in the art (Ausubel et al., supra, Sambrook et al., supra). Following transfer and UV cross linking, the membrane is hybridized with a radiolabeled probe in hybridization solution (e.g. in 50% formamide/2.5% Denhardt's/100-200mg denatured salmon sperm DNA/0.1% SDS/5X SSPE) at 42°C. The hybridization conditions can be varied as necessary as described in Ausubel et al., supra and Sambrook et al., supra. Following hybridization, the membrane is washed at room temperature in 2X SSC/0.1% SDS, at 42°C in 1X SSC/0.1% SDS, at 65°C in 0.2X SSC/0.1% SDS, and exposed to film. The stringency of the wash buffers can also be varied depending on the amount of background signal (Ausubel et al., supra).

#### RNase Protection Analysis

15 RNase Protection analysis can be used to analyze RNA structure and amount and determine the endpoint of a specific RNA.

The method of RNase protection is more sensitive than S1 analysis since it utilizes a sequence specific hybridization probe that is labeled to a high specific activity. The probe is hybridized to sample RNAs and treated with ribonuclease to remove free probe. Following ribonuclease treatment, the fragments comprising probe annealed to homologous sequences in the sample RNA are recovered by ethanol precipitation, and analyzed by electrophoresis on a sequencing gel. The presence of the target mRNA is indicated by the presence of an appropriately sized fragment of the probe.

A probe is labeled by the method of *in vitro* transcription (in the presence of [ $\alpha$ - $^{32}$ P] CTP as described in Section B entitled "Production of a Polynucleotide Sequence". The RNA sample to be analyzed is ethanol precipitated and resuspended in 30ml hybridization buffer (4 parts formamide/1 part 200 mM PIPES, pH 6.4, 2 M NaCl, 5 mM EDTA) containing  $5 \times 10^5$  cpm of the probe RNA. The mixture is denatured 5 minutes at 85°C and incubated at the desired hybridization temperature (30°C to 60°C) for >8 hours. To each reaction mixture is added 350  $\mu$ l ribonuclease digestion buffer (10 mM Tris-Cl, pH 7.5, 300 mM NaCl, 5 mM EDTA) containing 40 mg/ml ribonuclease A and 20 mg/ml ribonuclease T1. The sample is incubated for 30-60 minutes at 30°C. Following the addition of 10 ml 20% SDS and 2.5ml 20 mg/ml proteinase K, the sample is incubated for 15 minutes at 37°C. The sample is extracted with phenol /chloroform/isoamyl alcohol, ethanol precipitated, resuspended in RNA loading buffer (80% (v/v) formamide, 1 mM EDTA, pH 8.0, 0.1 % bromophenol blue, 0.1 % xylene

cyanol), denatured and analyzed by electrophoresis on a denaturing polyacrylamide/urea gel and autoradiography (Ausubel et al., supra).

#### Primer Extension

5           The method of primer extension is used to map the 5' end of an RNA and to quantitate the amount of an RNA of interest by using reverse transcriptase to extend a primer that is complementary to a region of a given RNA.

          An oligonucleotide primer is labeled in a kinase reaction as described for S1 analysis. The primer extension reaction is performed by mixing 10-50 mg total cellular RNA (in 10ml) with 1.5ml  
10   10X Hybridization buffer (1.5M KCl, 0.1M TrisCl, pH 8.3, 10mM EDTA) and 3.5 ml labeled oligonucleotide. Samples are heated to 65°C for 90 minutes and allowed to slow cool at room temperature. To each sample is added 30 ml of primer extension reaction mixture (0.9 ml Tris-Cl, pH 8.3, 0.9 ml 0.5M MgCl<sub>2</sub>, 0.25 ml DTT, 6.75 ml 1 mg/ml actinomycin D, 1.33 ml 5 mM 4dNTP mix, 20 ml H<sub>2</sub>O, 0.2ml 25 U/ml AMV reverse transcriptase). Samples are incubated for 1 hour at 42°C, and  
15   then, following the addition of 105 ml RNase reaction mix (100 mg/ml salmon sperm DNA, 20 mg/ml RNase A) for 15 minutes at 37°C. Samples are extracted in phenol/chloroform/isoamyl alcohol, ethanol precipitated, resuspended in stop/loading dye (20 mM EDTA, pH 8.0, 0.05% bromophenol blue, 0.05% xylene cyanol in formamide), heated at 65°C and analyzed by electrophoresis on a 9% acrylamide/7M urea gel and autoradiography.

#### In Situ Hybridization

          Cytological techniques well known in the art can be used to determine the temporal and spatial expression patterns of mRNA (*in situ* hybridization of tissue sections) and protein (immunohistochemistry in individual cells).

#### Preparation of histological samples

          Tissue samples intended for use in *in situ* detection of either RNA or protein are fixed using conventional reagents; such samples may comprise whole or squashed cells, or sectioned tissue. Fixatives useful for such procedures include, but are not limited to, formalin, 4% paraformaldehyde in  
30   an isotonic buffer, formaldehyde (each of which confers a measure of RNAase resistance to the nucleic acid molecules of the sample) or a multi-component fixative, such as FAAG (85 % ethanol, 4% formaldehyde, 5% acetic acid, 1% EM grade glutaraldehyde). For the detection of RNA, water used in the preparation of an aqueous component of a solution to which the tissue is exposed until it is

embedded is RNAase-free, i.e. treated with 0.1% diethylprocarbonate (DEPC) at room temperature overnight and subsequently autoclaved for 1.5 to 2 hours. Tissue will be fixed at 4°C, either on a sample roller or a rocking platform, for 12 to 48 hours in order to allow the fixative to reach the center of the sample.

5 Prior to embedding, excess fixative will be removed and the sample will be dehydrated by a series of two- to ten-minute washes in increasingly high concentrations of ethanol, beginning at 60% and ending with two washes in 95% and another two in 100% ethanol, followed by two ten-minute washes in xylene. Samples will be embedded in one of a variety of sectioning supports, e.g. paraffin, plastic polymers or a mixed paraffin/polymer medium (e.g. Paraplast®Plus Tissue Embedding  
10 Medium, supplied by Oxford Labware). For example, fixed, dehydrated tissue will be transferred from the second xylene wash to paraffin or a paraffin/polymer resin in the liquid-phase at about 58°C. The paraffin or a paraffin/polymer resin will be replaced three to six times over a period of approximately three hours to dilute out residual xylene. The sample will be incubated overnight at 58°C under a vacuum, in order to optimize infiltration of the embedding medium into the tissue. The next day,  
15 following several additional changes of medium at 20 minute to one hour intervals, also at 58°C, the tissue sample will be positioned in a sectioning mold, the mold will be surrounded by ice water and the medium will be allowed to harden. Sections of 6mm thickness will be taken and affixed to 'subbed' slides, which are slides coated with a proteinaceous substrate material, usually bovine serum albumin (BSA), to promote adhesion. Other methods of fixation and embedding are also applicable for use  
20 according to the methods of the invention; examples of these are found in Humason, G.L., 1979, Animal Tissue Techniques, 4th ed. (W.H. Freeman & Co., San Fransisco), as is frozen sectioning (Serrano et al., 1989, supra).

#### In situ Hybridization Analysis

25 According to the method of *in situ* hybridization a specifically labeled nucleic acid probe is hybridized to cellular RNA present in individual cells or tissue sections. *In situ* hybridization can be performed on either paraffin or frozen sections. Depending on the desired sensitivity and resolution, either film or emulsion autoradiography can be utilized to detect the hybridized radioactive probe.

The following method of *in situ* hybridization is performed by incubating slides containing cell  
30 or tissue specimens in a slide rack contained within a glass staining dish. According to this method, it is preferable to use solutions that have been prepared fresh. Prior to the hybridization steps, slides are dewaxed to remove the sectioning support material. The dewaxing protocol involves sequential washes in xylene, rehydration by sequential washes in 100%, 95%, 70% and 50% ethanol, and

denaturation in 0.2N HCl. Following a heat denaturation step (70°C in 2X SSC), samples are postfixed in a freshly prepared solution of 4% PFA, washed in PBS, incubated in 10 mM DTT (10 min at 45°C) and blocked in 400 ml PBS containing 0.617g DTT, 0.74 g iodoacetamide and 0.5g N-ethylmaleimide, for 30 min at 45°C in a water bath covered with aluminum foil, due to the light sensitivity of

5 iodoacetamide and N-ethylmaleimide. The samples are washed in PBS and equilibrated sequentially in freshly prepared 0.1M triethanolamine (TEA buffer), TEA buffer/0.25% acetic anhydride, and TEA buffer/0.5% acetic anhydride. Following a blocking step in 2X SSC, the sample are dehydrated by sequential washes in 50%, 70%, 95%, and 100% ethanol and air dried. <sup>35</sup>S-labeled riboprobes and competitor probes prepared in the absence of a radiolabel (prepared as described in Section B entitled

10 "Production of a Polynucleotide Sequence") or double-stranded DNA probes (prepared with [<sup>35</sup>S]dNTPs by methods well known in the art including nick translation or random oligonucleotide-primed synthesis) are heated to 100°C for 3 min and diluted to a concentration of 0.3 mg/ml final probe concentration, in 50% formamide, 0.3M NaCl, 10mM TrisCl, pH 8.0, 1 mM EDTA, 1x Denhardt solution, 500 mg/ml yeast tRNA, 500 mg/ml poly(A) (Pharmacia), 50 mM DTT, 10% polyethylene

15 glycol (MW 6000). The hybridization step is carried out by covering the sample with an appropriate amount of probe, and incubating for 30 min to 4 hour at 45°C in a chamber designed to prevent dilution or concentration of the hybridization solution. Samples are washed sequentially at 55°C in solution A (50% (v/v) formamide, 2X SSC, 20 mM 2-mercaptoethanol), and solution B (50% (v/v) formamide, 2X SSC, 20 mM 2-mercaptoethanol, 0.5% (v/v) Triton-X-100) and at room temperature in solution C

20 (2X SSC, 20 mM 2-mercaptoethanol). Following a 15 minute incubation with RNase, samples are washed at 50°C in solution C, and at room temperature in 2X SSC. Samples are rehydrated by sequential washes in 50% ethanol/0.3M ammonium acetate, 70% ethanol/0.3M ammonium acetate, 95% ethanol/0.3M ammonium acetate, and 100% ethanol. Slides are air dried and analyzed by film or by emulsion autoradiography (Ausubel et al., supra).

25

### iii. mRNA Stability/Control of Turnover and mRNA Transcription Rate

Changes in mRNA stability/control of turnover and mRNA transcription rates due to the presence of a polymorphism, can be detected by the following methods.

30

#### mRNA Stability

Gene-expression can be regulated by variations in mRNA stability (Liebhaber, 1997, Nucleic Acids Symp Ser., 36:29 and Ross J. 1996, Trends Genet., 5:171). Any gene variation occurring within



the cis-acting elements which control mRNA abundance may influence gene expression levels (Peltz et al., 1992, Curr Opin Cell Biol., 4:979). Quantitative RT-PCR (Kohler, et al, 1995, Quantitation of mRNA by polymerase chain reaction, Springer) and mRNA radiolabelling techniques are two methods for measuring relative mRNA abundance and stability. Quantitative PCR employs an internal standard to provide a direct comparison between alternative reactions, enabling comparison of low abundance transcripts or transcripts derived from a sample that is only available in a limited quantity (McPherson MJ et al., eds, 1995, PCR2- A practical approach. IRL Press).

#### Assay for mRNA Transcription Rates

Genetic polymorphism within the regulatory regions of a gene can significantly alter transcription rate and mRNA stability, resulting in reduced biological activity of the encoded protein. One of the most sensitive assays for measuring the rate of gene transcription is the nuclear runoff assay (Groudine and Casimir, 1984, Nucleic Acids Res 12: 1427). Nuclei isolated from cell lines expressing the target gene of interest are treated with radiolabelled UTP and the level of incorporation of radiolabel into nascent RNA transcripts is determined by filter hybridization to immobilized cDNA derived from the target gene.

#### iv. Intracellular mRNA Localization

A genetic variation can cause a change in the localization of a particular mRNA species (e.g. to the cytoskeleton, or to the nuclear scaffold).

#### Immunohistochemistry

Changes in RNA localization can be detected by immunohistochemical methods well known in the art (e.g. *in situ* analysis described above).

#### Oocyte Injection Assays

In many cases mRNA, like protein, is localized in relation to the polarity of the cell or the cytoskeletal architecture (St. Johnston, 1995, Cell, 81:161). The *Xenopus* oocyte is a popular, experimentally tractable, system for studying intracellular trafficking of mRNA (Nakielnny et al., 1997, Annu. Rev. Neurosci., 20:269). Fluorescently labelled RNA is microinjected into the large oocyte cell where its location can be detected using standard microscopy methods. Polymorphic variants of a particular mRNA species may differ in their response to cellular mechanisms responsible for partitioning mRNA within the cell. This method has been useful for demonstrating that sequence

variations can affect sub-cellular localization (Grimm et al., 1997, EMBO J., 16:793)

#### v. Post-Translational Alterations

Post-Translational alterations resulting from premature stop codons, translational readthrough  
5 or multiple open reading frames and translational suppression may occur as a result of a  
polymorphism. To detect post-translational alterations, a polynucleotide comprising one or more  
polymorphisms is subjected to *in vitro* transcription and *in vitro* translation (as described in sections B  
and J entitled "Production of a Polynucleotide Sequence" and "Preparation of a Labeled Protein").  
The translation product(s) are analyzed for the appearance of aberrantly sized proteins. Additional  
10 post-translational alterations that may occur as a result of a polymorphism include changes in  
localization due to an altered signal sequence, and changes in glycosylation, myristilation, and  
susceptibility to or sites of proteolytic cleavage.

The method of immunocytochemistry can be used to determine if a protein is incorrectly  
localized, due to the presence of an altered signal sequence.

15

#### Immunohistochemistry

Immunohistochemical techniques including indirect immunofluorescence, immunoperoxidase  
labeling or immunogold labeling, are used for protein localization.

Immunofluorescent labeling of tissue sections (prepared as for *in situ* analysis, described  
20 above) is performed by the following method. Slides containing the sample of interest are equilibrated  
to room temperature washed in PBS, incubated with an appropriate dilution of primary antibody (1  
hour at room temperature), washed in PBS, incubated with an appropriate dilution of secondary  
antibody (1 hour at room temperature), washed in PBS and analyzed under a microscope (Ausubel et  
al., supra). Alternatively, the sensitivity of the immunohistochemical reaction is increased by using a  
25 streptavidin-secondary antibody conjugate reacted with a biotinfluorochrome conjugate. Alternatively,  
immunogold labeling is used to detect a protein of interest by using an immunogold-conjugated  
secondary antibody.

Immunoperoxidase labeling of tissue sections is performed by the following method. Slides are  
pretreated in 0.25% hydrogen peroxide, incubated with primary antibody, washed in PBS and  
30 incubated (1 hour at room temperature) with a specific secondary bridging antibody capable of  
recognizing both the primary antibody and a Horseradish peroxidase antiperoxidase (PAP) complex.  
The slides are washed in PBS and developed in diaminobenzidine substrate solution (0.03% (w/v) 3,3'  
diaminobenzidine in 200 ml PBS) at room temperature (Ausubel et al., supra).

Alternatively, protein localization is determined by cell fractionation wherein cells are biosynthetically labeled, the labeled material is fractionated, and the radiolabeled proteins in each fraction are analyzed by immunoprecipitation with an antibody specific for the protein of interest.

#### 5 Assay for Glycosylation Inhibition

Changes in protein glycosylation can be detected by radiolabelling a protein of interest with sugars, determining if a change in the cellular localization (by immunocytochemistry) of the protein in culture has occurred due to aberrant glycosylation, or by determining the effects of inhibitors of glycosylation on the migration pattern of proteins analyzed by polyacrylamide gel electrophoresis.

10 Post-translational glycosylation of proteins plays an important role in defining protein function (Baeziger, 1994, FASEB J., 13:1019; Jacob, 1995, Curr. Opin. Struct. Biol., 5:605). Protein glycosylation can be inhibited by tunicamycin, an antibiotic, as well as by several sugar analogues (Schwarz, 1991, Behring Inst Mitt., 89:198). These reagents are used to characterize the effects of sequence changes on protein glycosylation.

15

#### Assay for Post-Translational Modification with Lipids

Changes in protein modification with lipids (e.g. myristilation) are detected by radiolabelling a protein of interest with myristic acid or by determining if a change in the cellular localization of the protein in culture has occurred as a result of aberrant lipid modification (by immunocytochemistry).

20 Covalent attachment of lipids is a mechanism by which eukaryotic cells direct and, in some cases, control, membrane localization of proteins (Casey, 1994, Curr. Opin. Cell. Biol., 2:219). Such post-translational addition of myristyl, palmityl or prenyl side-chains has a key role in the functional regulation of many proteins (Chow et al., 1992, Curr. Opin. Cell. Biol., 4:629; Resh, 1994, Cell, 763:411). Assays for detecting proteins that are covalently modified by the attachment of lipids include  
25 labeling with [<sup>3</sup>H]myristate (Stevenson et al., 1992, J. Exp. Med., 176:1053), or a combination of enzymatic and chemical cleavage techniques performed in conjunction with tandem mass spectrometry to determine sites of modification (Papac et al., 1992, J. Biol. Chem., 267:16889).

#### Proteolytic Cleavage

30 Post-translational cleavage of polypeptides is an important mechanism for modulating protein function in many physiological processes. Protease activity is involved in zymogen processing, activation of enzyme catalysis, tissue/cell remodeling, signal transduction cascades, protein degradation and cell death pathways (Rappay, 1989, Prog Histochem Cytochem., 18:1). A protein that is predicted

to be a protease or the target of a protease can be assayed *in vitro* using purified proteins or cell extracts (Muta et al., 1995, J. Biol. Chem. 270:892) where cleavage efficiency is monitored by standard PAGE or western blotting. Alternatively, proteases and/or their targets can be expressed from expression plasmids in *in vivo* cell culture systems in order to monitor their biological activity (Zhang, et al., 1998, J. Biol. Chem. 273:1144). The specificity of proteolytic cleavage is determined using inhibitors that selectively block serine, cysteine, aspartic and metallo proteolytic activity (e.g. pepstatin A selectively inhibits aspartic proteases) (Rich, et al., 1985, Biochemistry., 24: 3165).

To determine if a protein has been modified such that the sites of proteolytic cleavage have been altered, or susceptibility to proteolytic cleavage has changed pulse chase experiments with radiolabeled protein can be carried out to determine the precursor-product relationship following digestion with a protease of a given specificity. The method of pulse chase labeling is described in Ausubel et al., supra. Alternatively, inhibitors of proteases (e.g acid proteases or serine proteases) can be used to identify protease cleavage sites.

#### vi. Changes in Receptor Properties

If the gene of interest encodes a receptor protein, a polymorphism may modify the properties of the receptor such that receptor binding/turnover or activation is altered. Receptor formation can be impaired if a polymorphism causes improper receptor localization or assembly.

#### Receptor Localization

To determine if a receptor protein is being expressed at the proper location (e.g. nucleus, cytoplasm, cell surface), the receptor can be localized by immunocytochemical techniques. Alternatively, cells that are expressing the receptor can be fractionated and subjected to Western blot analysis or biosynthetically labeled, fractionated and analyzed by immunoprecipitation.

#### Protein-Protein Interactions/*In vitro* Assembly Assays for Receptors

A number of methods can be used to determine if a receptor is colocalized with the appropriate protein partner.

The function of a protein may be dependent on the ability of the protein to interact with other proteins as part of a large complex. For example, certain cell surface receptors consist of a receptor complex that is composed of several homo- or heteromeric protein subunits, and activation by ligand can result in altered protein-protein interactions both within the receptor complex and with “downstream” targets such as G-proteins (Okada and Pessin, 1996, J. Biol. Chem., 271:25533).

Protein-protein interactions can be assayed immunologically by coimmunoprecipitation of native (Gilboa et al., 1998, J. Biol. Chem., 140:767) or chemically cross-linked complexes (Haniu et al., 1997, J. Biol. Chem., 272:25296), or through protein-protein mobility shift assays (Stern and Frieden, 1993, Anal. Biochem., 212:221). If all of the components of a receptor complex have been identified, one  
5 can employ *in vitro* reconstitution assays to assess whether a single protein alteration can effect the functioning of the entire complex (Durovic et al., 1994, J. Biol. Chem., 269:30320).

#### Assay for *In Vitro* Assembly of Multimeric Protein Complexes

To determine whether these genetic variations have affected protein complex assembly,  
10 experiments are carried out wherein recombinant mutant subunits are transfected into cells and coexpressed with the other subunit components *in vitro*. Proper assembly is assessed by immunoprecipitation of the protein complex in question with antibodies specific for the various members of the complex followed by PAGE analysis (Koster et al., 1998, Biophysl. J., 74:1821).

#### Assay Receptor Binding/Turnover

Receptor-ligand interaction is essential for the functionality of the bound complex. Genetic  
changes that alter either ligand or receptor can dramatically affect receptor binding, turnover, and  
subsequent activation of downstream signaling events. Receptor binding/turnover can be measured by  
standard Scatchard analysis of radiolabelled ligand binding *in vitro* (Culouscou et al., 1993, J. Biol.  
20 Chem. 268:10458) or in cellular based assays (Greenlund et al., 1993, J. Biol. Chem. 268: 18103).

#### Ligand Binding as Measured by Affinity Chromatography

Alternatively, affinity chromatography methods (well known in the art) can be employed to  
determine if a receptor is demonstrating aberrant binding characteristics. According to the method of  
25 affinity chromatography, receptor-ligand interactions are allowed to occur, and the binding efficiency  
or receptor and ligand and/or turnover of receptor-ligand complexes is measured. Alternatively,  
affinity chromatography can be used to isolate one or more components of a receptor ligand  
interaction for further analysis (March et al., 1974, Adv. Exp. Med. Biol., 42:3). The method of affinity  
chromatography typically involves immobilizing on a solid support one component, for example a  
30 known ligand for a receptor, and then incubating the immobilized ligand with radiolabelled protein  
under optimal binding conditions. To measure the exact binding affinity of a given ligand-receptor pair,  
an increasing amount of non-labeled competitor is added. This assay can be used to assess altered  
binding efficiency resulting from the presence of a polymorphism in a protein of interest.

### Receptor Activation Assays: Phosphorylation, Kinase Activity and Mitogenic Stimulation

Almost all signaling that occurs through cell surface receptors is regulated by phosphorylation, a reversible post-translational event that occurs at specific amino acid residues and is catalyzed by a protein kinase activity present within the receptor itself (autophosphorylation) or in trans via direct  
5 interaction with an associated kinase (Hunter, 1997, *Philos Trans R Soc Lond B Biol Sci.*, 353:583). The specific effect of phosphorylation on a biological activity depends on the receptor, but often results in modulation of endogenous receptor kinase activity or interaction with associated proteins, which are also often kinases. The results of a phosphorylation event are passed on through a cascade of protein kinases/phosphatases which ultimately effect downstream processes controlling gene  
10 transcription, cell proliferation, metabolism, movement and differentiation (Patarca, 1996, *Crit Rev Oncog.*, 7:343). The biological function of a receptor is usually assayed in cell culture following over-expression. The phosphorylated state of a receptor can be assayed directly by immunological methods by employing an antibody that specifically recognizes a phosphorylated residue (Bangalore, 1992., *Proc Natl Acad Sci USA.*, 89:11637). Endogenous kinase activity associated with a receptor is  
15 measured via the incorporation of radiolabelled phosphate in immunoprecipitated receptor complex (Kazlauskas and Cooper, 1989, *Cell* 58:1121). "Downstream" events of receptor activity including mitogenic stimulation or map kinase activity, can be measured by tritiated thymidine incorporation (Luo et al., 1996, *Cancer Res.* 56:4983), or by mobility-shift analysis of map kinase on western blots (Vietor, 1993., *J. Biol. Chem.* 268:18994), respectively.

20 Immunocytochemical methods can be used to determine if a receptor-ligand complex is correctly translocated to the nucleus. Alternatively, nuclear preparations (prepared as described below) can be analyzed by Western blot or immunoprecipitation for the presence of the receptor protein.

If a receptor is a transcriptional activator, the ability of the receptor to induce gene expression  
25 can be measured by a variety of methods including Northern blot analysis, or reporter gene assays wherein the promoter region isolated from a gene that is activated by the receptor regulates the expression of a reporter protein.

#### vii. Enzyme Catalysis

30 The gene of interest may encode a protein that has an enzymatic activity wherein the enzyme catalyzes a reaction that is critical to the general metabolism of a cell. To determine if a mutated protein is impaired in its enzymatic function, assays can be performed to measure the enzymatic activity of the protein. There are many important enzymatic activities associated with normal cellular

metabolism, including: glycosidation, esterification, amidation, hydroxylation, acetylation, sulfonylation, alkylation. Each of these activities are assayed using *in vitro* methods employing overexpressed or purified proteins, well known in the art (Eisenthal and Danson, 1992, Enzyme Assays: A Practical Approach, Rickwood et al., Eds., IRL Press. Oxford, England).

5           The protein of interest may also be involved in various aspects of DNA synthesis or replication. *In vitro* assays for the enzymatic reactions involved in DNA synthesis or replication (e.g. polymerase, ligase, exonuclease or helicase activity) are known in the art. The biological activity of the proteins catalyzing these activities are assayed *in vitro* using standard enzymatic techniques (Adams, 199, DNA Replication: A Practical Approach I, Rickwood, et al., Eds., IRL Press. Oxford, England).

10           If the protein of interest is involved in glycolysis or energy transport, assays for measuring transporter activity or the activity of ATP dependent pumps are useful, according to the invention, for determining if a mutated protein is impaired in these functions.

#### Transporter Activity

15           Mammalian cells possess a variety of transporter systems, for example amino acid transporters, which have overlapping substrate specificity (Van Winkle et al., 1993, Biochim Biophys Acta, 1154:157). To determine if a polymorphism in a candidate gene of interest has altered the function of the protein product of this gene as a molecular transporter, the full-length cDNA clone is isolated by standard expression cloning strategies, and a change in activity of the full-length cRNA or  
20           antisense cRNA upon microinjection into *Xenopus laevis* oocytes is determined by measuring changes in influx/efflux transport of radiolabelled amino acid molecules (Broer et al., 1995, Biochem J., 312(Pt 3):863), neurotransmitters or their metabolites.

#### ATP-dependent pumps Activity

25           Mammalian cells possess a variety of molecules that are categorized as ATP-binding cassette or ATP-dependent transporters or pumps. These include the Na<sup>+</sup>-K<sup>+</sup>-ATPase ion pump, the calcium uptake pump, (K<sup>+</sup> + H<sup>+</sup>)-ATPase and the human multidrug resistant protein termed P-glycoprotein. Alterations in pump activity are investigated by expressing the clone specific for the pump protein(s) of interest in *Xenopus* oocytes, and performing tracer studies which measure the changes in ATP-  
30           dependent uptake or extrusion of a radiolabelled substrate, and changes in the coupling ratios (e.g. moles substrate transported/mole ATP hydrolyzed) (Shapiro et al., 1998, Eur. J. Biochem., 254:189).

The gene of interest may encode for a protein that is a component of an ion channel. Immunocytochemical methods can be used to determine if an ion channel protein demonstrates the appropriate cell type specificity.

The activity of an ion channel can be measured by electrophysiological methods in oocytes.

5 Alternatively, the sensitivity of ion channel activity to a particular inhibitor can be determined.

#### Assays for Ion Channel Activity in Oocytes

Polymorphisms which alter ion channel function and regulation are studied using the oocytes of *Xenopus laevis*. Injection of the oocytes with exogenous *in vitro* transcribed mRNA results in the  
10 production and functional expression of foreign membrane proteins, including voltage- and neurotransmitter- operated ion channels (Dascal et al., 1987., CRC Crit Rev Biochem., 224:317). Changes in the oocyte transmembrane current in response to expression of an exogenous mRNA is measured. This technique has been improved by the development of rapid superfusion systems that utilize a dual role perfusion micropipette that controls internal solution as well as monitoring voltage  
15 (Costa et al., 1994, Biophys J., 67:395). This technology represents a useful system for studying various aspects of ion channels encoded for by foreign mRNAs including channel expression, single-channel behavior, and the response of channels to the action of pharmacologically active substances (Sigel, 1987, J. Physiol., 386: 73).

#### 20 Patch Clamp Assays for Ion Channel Activity

The function of individual channel proteins is determined by the high resolution patch clamp technique. This technique (which is useful in a variety of cell types, including *Xenopus* oocytes described above) involves measuring changes in transmembrane current across the cell membrane *in vitro* (Sachs et al., 1983, Methods Enzymol., 103: 147). Processes such as signaling, secretion, and  
25 synaptic transmission are examined at the cellular level by the patch clamp method. The gene expression pattern and protein structure of ionic channels can be determined by combining information derived from high-resolution electrophysiological recordings obtained by the patch clamp method with molecular biological analysis (Liem et al., 1995, Neurosurgery, 36: 382).

A polymorphic variation in a gene that encodes a protein that is a member of a multimeric  
30 protein complex, such as an ion channel or a cytoskeletal structural component, can alter the assembly and function the multimeric protein complex (Lee et al., 1994., Biophys J., 66: 667). A gene variation may affect protein-protein interaction, or disrupt the production of components of a multimeric complex, thereby disrupting stoichiometry and consequently decreasing stability.



### Assay for *In Vitro* Assembly of Multimeric Protein Complexes

*In vitro* assembly assays (described above) can be performed to determine if a polymorphism has affected the assembly of an ion channel.

#### 5           ix.       Cellular Properties

The influence of a polymorphism on general aspects of cell behavior, including cell morphology, adhesive properties, differentiation and proliferation can be assessed using a combination of methods including microscopic observation of cell cultures (Azuma et al., 1994, *Histol.Histopathol.*, 9:781), immunohistochemistry, and FACs analysis techniques (Beesley, 1993, *Immunocytochemistry: a*  
10 *Practical Approach*, Rickwood, et al., (Eds), IRL Press and Ormerod, 1994, *Flow Cytometry: a practical Approach*, Rickwood et al., (Eds), IRL Press. Oxford, England).

### Assays for Measuring Apoptosis

Apoptosis has been implicated in the etiology and pathophysiology of a variety of human  
15 diseases. Gene variants which influence the process of apoptosis can be assessed by a variety of methods of analysis involving either the tissues or cells (Allen et al., 1997, *J Pharmacol Toxicol Methods*, 37: 215). Cell cultures expressing the gene variants of interest are analyzed using Annexin V which interacts strongly with phosphatidylserine residues that have been exposed as a result of plasma  
20 membrane breakdown occurring in the early stages of apoptosis. Either vital or fixed material can be analyzed by Annexin V labeling in combination with microscopy and flow cytometry detection methods (van Engeland et al., 1998, *Cytometry*, 31:1). TdT-mediated deoxyuridine triphosphate (dUTP)-biotin nick end-labeling (TUNEL) is a preferred method for specific staining of apoptotic cells in histological sections and cytology specimen (Labat-Moleur et al., 1998, *J. Histochem Cytochem.*, 46:327; Sasano et al., 1998., *Diagn Cytopathol.*, 18:398). Apoptosis is also detected by quantification of  
25 DNA fragmentation by ethidium bromide staining and gel electrophoresis, or by the use of saturation labeling of 3' ends of DNA fragments (Peng and Liu, 1997, *Lab Invest.*, 77:547).

### Assay for *In Vivo* Receptor Function: Growth Cone Guidance Assay

Activation of cell-surface receptors can result in the stimulation of cell motility. There are  
30 many different families of signaling molecules, for example the netrins, (Serafini et al., 1994, *Cell*. 78: 409), which are responsible for both contact mediated or chemo-mediated attraction and repulsion of migrating cells. A classic model for this activity is the trajectory that the leading edge "growth cone" takes when a neuron is stimulated to grow out from explanted neural tissue in cell culture (Goodman,

1996, Annu Rev Neurosci. 19: 341). Ligands present in the culture medium or immobilized on a substrate bind to receptors on the cell-surface of the growth cone and trigger second-messenger signals thereby dictating an appropriate steering response. The biological activity of such receptors or ligands can be measured by overexpressing the receptor or ligand protein in culture and then  
5 monitoring growth cone guidance (Kremoser et al., 1995, Cell 82: 359). Attraction or repulsion of cells which is observed to be different than normal is an indication of the role of this protein in growth guidance, and identifies the polymorphisms as altering function.

x. Changes in gene expression or protein function that result from the presence of a  
10 polymorphism can be detected by *in vivo* assays including the production of transgenic animals, knock out animals or the analysis of naturally occurring animal models of a particular disease.

#### Transgenic Animals

Transgenic mice provide a useful tool for genetic and developmental biology studies and for  
15 the determination of a function of a novel sequence. According to the method of conventional transgenesis, additional copies of normal or modified genes are injected into the male pronucleus of the zygote and become integrated into the genomic DNA of the recipient mouse. The transgene is transmitted in a Mendelian manner in established transgenic strains.

Constructs useful for creating transgenic animals comprise genes under the control of either  
20 their normal promoters or an inducible promoter, reporter genes under the control of promoters to be analyzed with respect to their patterns of tissue expression and regulation, and constructs containing dominant mutations, mutant promoters, and artificial fusion genes to be studied with regard to their specific developmental outcome. Transgenic mice are useful according to the invention for analysis of the dominant effects of overexpressing a candidate gene in mouse. Typically, DNA fragments on the  
25 order of 10 kilobases or less are used to construct a transgenic animal (Reeves, 1998, New. Anat., 253:19). Transgenic animals can be created with a construct comprising a candidate gene containing one or more polymorphisms according to the invention. Alternatively, a transgenic animal expressing a candidate gene containing a single polymorphism can be crossed to a second transgenic animal expressing a candidate gene containing a different polymorphism and the combined effects of the two  
30 polymorphisms can be studied in the offspring animals. Transgenic mice engineered to overexpress a number of genes, including PCK1 (Valera et al., 1994, Proc. Natl. Acad. Sci. USA, 91: 9151), INS (Mitanchéz et al., FEBS Letters, 421: 285), IAPP (D'Alession et al., 1994, Diabetes, 43:1457), Asp (Klebig et al., Proc. Natl. Acad. Sci. USA, 92: 4728) and Agrt (Graham et al., Nature Genetics,

17:273), have been prepared and may be useful for studying osteoarthritis.

### Knock Out Animals

#### i. Standard

Knock out animals are produced by the method of creating gene deletions with homologous recombination. This technique is based on the development of embryonic stem (ES) cells that are derived from embryos, are maintained in culture and have the capacity to participate in the development of every tissue in the mouse when introduced into a host blastocyst. A knock out animal is produced by directing homologous recombination to a specific target gene in the ES cells, thereby producing a null allele of the gene. The potential phenotypic consequences of this null allele (either in heterozygous or homozygous offspring) can be analyzed (Reeves, supra). Single or double knock out mice that may be useful for studying osteoarthritis have been produced for a number of genes including IRS 1 (Araki et al., 1994, Nature, 372:186, Tamemoto et al., 1994, Nature, 372:182), 1R52 (Withers et al., 1998, Nature, 391:900), INSR, BIRKO, MIRKO, INSR (Lamothe et al., 1998, FEBS Letter, 426:381), GLUT2, GLUT4 (Katz et al., 1995, Nature, 377:151), GLP1R (Gallwitz and Schmidt, 1997, Z. Gastroenterol, 35:655), GCK (Sakura et al., 1998, Diabetologia, 41:654), GCK/IRS1, IRS1/INSR, MC4R (Huszar et al., 1997, Cell, 88:13 1) and BRS3 (Ohki-Hamazaki et al., 1997, Nature, 390:165).

#### ii. *In vivo* Tissue Specific Knock Out in Mice Using Cre-lox.

The method of targeted homologous recombination has been improved by the development of a system for site-specific recombination based on the bacteriophage P1 site specific recombinase Cre. The Cre-loxP site-specific DNA recombinase from bacteriophage P1 is used in transgenic mouse assays in order to create gene knockouts restricted to defined tissues or developmental stages. Regionally restricted genetic deletion, as opposed to global gene knockout, has the advantage that a phenotype can be attributed to a particular cell/tissue (Marth, 1996, Clin. Invest. 97: 1999). In the Cre-loxP system one transgenic mouse strain is engineered such that loxP sites flank one or more exons of the gene of interest. Homozygotes for this so called 'floxed gene' are crossed with a second transgenic mouse that expresses the Cre gene under control of a cell/tissue type transcriptional promoter. Cre protein then excises DNA between loxP recognition sequences and effectively removes target gene function (Sauer, 1998, Methods, 14:381). There are now many *in vivo* examples of this method, including the inducible inactivation of mammary tissue specific genes (Wagner et al.,

1997, Nucleic Acids Res., 25:4323).

### iii. Bac Rescue of Knock Out Phenotype

In order to verify that a particular genetic polymorphism/mutation is responsible for altered protein function *in vivo* one can “rescue” the altered protein function by introducing a wild-type copy of the gene in question. *In vivo* complementation with bacterial artificial chromosome (BAC) clones expressed in transgenic mice can be used for these purposes. This method has been used for the identification of the mouse circadian Clock gene (Antoch et al., 1997, Cell 89: 655).

### iv. Naturally Occurring Animal Models

Naturally occurring animal models useful for studying osteoarthritis include models of severe hyperglycaemia (celebes black ape, chinese hamster, diabetes mouse (db), Djunjarian hamster, Egyptian sand rat, Hartley guinea pig, OLETF rat, New Zealand white rabbit, obese *BBZ/Wor* rat, rhesus monkey, South African hamster, spiny mouse), models for moderate hyperglycaemia (Cohen diabetic rat, GK rat, Japanese KK mouse, male Bristol CBA/Ca mouse, male eSS rat, male WKY fatty rat, male Wistar WBN/Kob rat, male ZDF rat, NZO mouse, obese mouse (ob), PBB/Ld mouse, spontaneously hypertensive corpulent (SHR/N-cp) rat, Tuco-tuco, Wellesley hybrid mouse, yellow obese mouse) and impaired glucose tolerance (ageing laboratory rats and mice, BHE rat, Fatty Zucker rat (fa), Mongolian gerbil, NON diabetic mouse, squirrel monkey, Yucatan miniature swine) (Pickup and Williams, eds., Textbook of Diabetes, 2nd Edition, Blackwell Science).

## G. Production of an Amplified Product

Amplified products useful according to the invention can be prepared by utilizing the method of PCR as described in Section B entitled “Production of a Polynucleotide Sequence Primers useful for producing an amplified product according to the invention (e.g. an amplified product comprising one or more polymorphisms) can be designed and synthesized as described in Section A entitled “Design and Synthesis of Oligonucleotide Primers”.

The invention provides methods (e.g. Southern blot analysis, PCR, primer extension and oligonucleotide hybridization), of detecting a polymorphism in an amplified product.

## H. Production of a Mutant Protein

### 1. Expression of the Nucleotide Sequence

In accordance with the present invention, polynucleotide sequences which encode candidate gene protein fragments, fusion proteins or functional equivalents thereof may be used in recombinant DNA molecules that direct the expression of a candidate gene protein in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence, may be used to clone and express the candidate gene protein. As will be understood by those of skill in the art, it may be advantageous to produce candidate gene-encoding nucleotide sequences possessing non-naturally occurring codons. Codons preferred by a particular prokaryotic or eukaryotic host (Murray et al., 1989, Nucleic Acid Res 17:477) can be selected, for example, to increase the rate of protein expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life as compared to transcripts produced from the naturally occurring sequence.

The nucleotide sequences of the present invention can be engineered in order to alter a candidate gene-encoding sequence for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the gene product. For example, mutations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis to insert new restriction sites, to alter glycosylation patterns, to change codon preference or to produce splice variants.

In another embodiment of the invention, a natural, modified or recombinant candidate gene protein-encoding sequence may be ligated to a heterologous sequence to encode a fusion protein (as described in Section B entitled "Production of a Polynucleotide Sequence"). For example, for screening of peptide libraries for inhibitors of candidate gene protein activity, it may be useful to encode a chimeric protein that is recognized by a commercially available antibody. a fusion protein may also be engineered to contain a cleavage site located between a candidate protein and the heterologous protein sequence, so that the protein of interest may be substantially purified away from the heterologous moiety following cleavage.

In another embodiment of the invention, the sequence encoding the candidate gene protein may be synthesized, whole or in part, using chemical methods well known in the art (see Caruthers, et al., 1980, Nuc Acids Res Symp Ser, 7:215, Horn, et al., 1980, Nuc Acids Res Symp Ser, 225, etc.) Alternatively, the protein itself, or a portion thereof, could be produced using chemical methods of synthesis. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, et al., 1995, Science, 269:202) and automated synthesis may be achieved, for example, using the A.I. 431 A Peptide Synthesizer (Perkin Elmer) in accordance with the instructions provided by the manufacturer.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (e.g., Creighton, 1983, *Proteins, Structures and Molecular Principles*, WH Freeman and Co. New York NY). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; Creighton, *supra*).

5 Additionally the amino acid sequence of interest, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

## 2. Expression Systems

10 In order to express a biologically active protein, the nucleotide sequence encoding the protein of interest or its functional equivalent, is inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence.

Methods which are well known to those skilled in the art can be used to construct expression  
15 vectors containing a protein-encoding sequence and appropriate transcriptional or translational controls. These methods include *in vivo* recombination or genetic recombination. Such techniques are described in Ausubel et al., *supra* and Sambrook et al., *supra*.

A variety of expression vector/host systems may be utilized to contain and express a protein product of a candidate gene according to the invention. These include but are not limited to  
20 microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transfected with virus expression vector (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (e.g., Ti or pBR322 plasmid); or animal cell systems.

25 The "control elements" or "regulatory sequences" of these systems vary in their strength and specificities and are those nontranslated regions of the vector, enhancers, promoters, and 3' untranslated regions, which interact with host cellular proteins to carry out transcription and translation. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when  
30 cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the Bluescript® phagemid (Stratagene, LaJolla CA) or pSport1 (Gibco BRL) and ptrp-lac hybrids and the like may be used. The baculovirus polyhedron promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO; and storage protein genes) or

from plant virus (e.g. viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems promoters from the mammalian genes or from mammalian viruses are most appropriate. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding the protein product of the gene of interest, vectors based on 5V40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for the protein of interest. For example, when large quantities of a protein are required for the production of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be desirable. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as Bluescript® (Stratagene), in which the sequence encoding the protein of interest may be ligated into the vector in frame with sequences encoding the amino-terminal Met and the subsequent 27 residues of b-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke & Schuster, 1989, J Biol Chem 264:5503); and the like. Pgex vectors (Promega, Madison WI) may also be used to express foreign polypeptides as fusion proteins with GST. In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems are designed to include heparmn, thrombin or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase and PGH may be used. For reviews, see Ausubel et al (supra) and Grant et al., 1987, Methods in Enzymology 153:516.

In cases where plant expression vectors are used, the expression of a sequence encoding a protein of interest may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV (Brisson et al., 1984, Nature 310:511) may be used alone or in combination with the omega leader sequence from TMV (Takamatsu et al., 1987, EMBO J 6:307). Alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi et al., 1984, EMBO J 3:1671; Broglie et al., 1984, Science, 224:838); or heat shock promoters (Winter I and Sinibaldi RM, 1991, Results Probl Cell Differ., 17:85) may be used. These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transection. For reviews of such techniques, see Hobbs S or Murry LE in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill New York NY, pp 191-196 or Weissbach and Weissbach (1988) Methods for Plant Molecular Biology, Academic Press, New York, pp 421-463.

An alternative expression system which could be used to express a protein of interest is an

insect system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. The sequence encoding the protein of interest may be cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of the sequence encoding the protein of interest will render the polyhedron gene inactive and produce recombinant virus lacking coat protein coat. The recombinant viruses are then used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which the protein of interest is expressed (Smith et al., 1983., J Virol 46:584; Engelhard, et al., 1994, Proc Natl Acad Sci 91:3224).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, a sequence encoding the protein of interest may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a nonessential E1 or E3 region of the viral genome will result in a viable virus capable of expressing in infected host cells (Logan and Shenk, 1984, Proc Natl Acad Sci, 81:3655). In addition, transcription enhancers, such as the rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be required for efficient translation of a sequence encoding the protein of interest. These signals include the ATG initiation codon and adjacent sequences. In cases where the sequence encoding the protein, its initiation codon and upstream sequences are inserted into the most appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon must be provided. Furthermore, the initiation codon must be in the correct reading frame to ensure transcription of the entire insert. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (Scharf, et al., 1994, Results Probl Cell Differ, 20:125; Bittner et al., 1987, Methods in Enzymol, 153:516).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, 293, W138, etc have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing



of the introduced, foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express a foreign protein may be transformed using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable  
5 marker gene. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clumps of stably transformed cells can be expanded using tissue culture techniques appropriate to the cell type.

10 Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223) and adenine phosphoribosyltransferase (Lowy, et al., 1980, Cell 22:817) genes which can be employed in tk- or apt- cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler  
15 et al., 1980, Proc Natl Acad Sci 77:3567); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin et al., 1981., J Mol Biol., 150:1) and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, supra). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman and  
20 Mulligan, 1988, Proc Natl Acad Sci 85:8047). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, B glucuronidase and its substrate, GUS, and luciferase and its substrate, luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes et al., 1995, Methods Mol Biol 55:121).

### 25 3. Identification of Transformants Containing the Polynucleotide Sequence

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression should be confirmed. For example, if the sequence encoding a foreign protein is inserted within a marker gene sequence, recombinant cells containing the  
30 sequence encoding the foreign protein can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with the sequence encoding the foreign protein under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem sequences as well.

Alternatively, host cells which contain the coding sequence for a protein of interest and express the protein of interest may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridization and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of the nucleic acid or protein.

The presence of the polynucleotide sequence encoding the protein of interest can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes, portions or fragments of the sequence encoding the foreign protein of interest.

A variety of protocols for detecting and measuring the expression of the foreign protein, using either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples include enzyme-linked immunosorbant assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on the protein of interest is preferred, but a competitive binding assay may be employed. These and other assays are described in Hampton et al., 1990, *Serological Methods a Laboratory Manual*, APS Presds, St Paul MN and Maddox., et al., 1983, *J Exp Med* 158:1211.

#### 4. Purification of the Protein of Interest

Host cells transformed with a nucleotide sequence encoding a protein of interest may be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing a sequence encoding a protein of interest can be designed with signal sequences which direct secretion of the protein of interest through a prokaryotic or eucaryotic cell membrane. Other recombinant constructions may join the sequence encoding the protein of interest to the nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins (Kroll et al., 1993, *DNA Cell Biol*, 12:441).

The protein of interest may also be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as a histidine-tryptophan modules that allow purification on immobilized metals, protein a domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle WA). The inclusion of a cleavable linker sequences such as Factor XA or enterokinase

(Invitrogen, San Diego CA), between the purification domain and the protein of interest is useful for facilitating purification. One such expression vector provides for expression of a fusion protein comprising the sequence encoding a foreign protein and nucleic acid sequence encoding 6 histidine residues followed by thioredoxin and an enterokinase cleavage site. The histidine residues facilitate purification while the enterokinase cleavage site provides a means for purifying the foreign protein from the fusion protein.

In addition to recombinant production, fragments of the protein of interest may be produced by direct peptide synthesis using solid-phase techniques (Stewart et al., 1969, Solid-Phase Peptide Synthesis, WH Freeman Co., San Francisco; Merrifield, 1963, J Am Chem Soc, 85:2149). *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431 A Peptide Synthesizer (Perkin Elmer, Foster City CA) in accordance with the instructions provided by the manufacturer. Various fragments of a protein of interest may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

## I. Preparation of Antibodies

Antibodies specific for the protein products of the candidate genes of the invention are useful for protein purification, for the diagnosis and treatment of various diseases (e.g. osteoarthritis) and for drug screening and drug design methods useful for identifying and developing compounds to be used in the treatment of various diseases (e.g. osteoarthritis). By antibody, we include constructions using the binding (variable) region of such an antibody, and other antibody modifications. Thus, an antibody useful in the invention may comprise a whole antibody, an antibody fragment, a polyfunctional antibody aggregate, or in general a substance comprising one or more specific binding sites from an antibody. The antibody fragment may be a fragment such as an Fv, Fab or F(ab')<sub>2</sub> fragment or a derivative thereof, such as a single chain Fv fragment. The antibody or antibody fragment may be non-recombinant, recombinant or humanized. The antibody may be of an immunoglobulin isotype, e.g., IgG, IgM, and so forth. In addition, an aggregate, polymer, derivative and conjugate of an immunoglobulin or a fragment thereof can be used where appropriate. Neutralizing antibodies are especially useful according to the invention for diagnostics, therapeutics and methods of drug screening and drug design.

Although a protein product (or fragment or oligopeptide thereof) of a candidate gene of the invention that is useful for the production of antibodies does not require biological activity, it must be antigenic. Peptides used to induce specific antibodies may have an amino acid sequence consisting of

at least five amino acids and preferably at least 10 amino acids. Preferably, they should be identical to a region of the natural protein and may contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of amino acids corresponding to the protein product of a candidate gene of the invention may be fused with amino acids from another protein such as keyhole limpet hemocyanin or GST, and antibody will be produced against the chimeric molecule. Procedures well known in the art can be used for the production of antibodies to the protein products of the candidate genes of the invention.

For the production of antibodies, various hosts including goats, rabbits, rats, mice etc... may be immunized by injection with the protein products (or any portion, fragment, or oligonucleotide thereof which retains immunogenic properties) of the candidate genes of the invention. Depending on the host species, various adjuvants may be used to increase the immunological response. Such adjuvants include but are not limited to Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. BCG (bacilli Calmette-Guerin) and *Corynebacterium parvum* are potentially useful human adjuvants.

#### 1. Polyclonal antibodies.

The antigen protein may be conjugated to a conventional carrier in order to increase its immunogenicity, and an antiserum to the peptide-carrier conjugate will be raised. Coupling of a peptide to a carrier protein and immunizations may be performed as described (Dymecki et al., 1992, J. Biol. Chem., 267: 4815). The serum can be titrated against protein antigen by ELISA (below) or alternatively by dot or spot blotting (Boersma and Van Leeuwen, 1994, J Neurosci. Methods, 51: 317). At the same time, the antiserum may be used in tissue sections prepared as described. A useful serum will react strongly with the appropriate peptides by ELISA, for example, following the procedures of Green et al., 1982, Cell, 28: 477.

#### 2. Monoclonal antibodies.

Techniques for preparing monoclonal antibodies are well known, and monoclonal antibodies may be prepared using a candidate antigen whose level is to be measured or which is to be either inactivated or affinity-purified, preferably bound to a carrier, as described by Arnheiter et al., 1981, Nature, 294:278.

Monoclonal antibodies are typically obtained from hybridoma tissue cultures or from ascites

fluid obtained from animals into which the hybridoma tissue was introduced.

Monoclonal antibody-producing hybridomas (or polyclonal sera) can be screened for antibody binding to the target protein.

### 3. Antibody Detection Methods

Particularly preferred immunological tests rely on the use of either monoclonal or polyclonal antibodies and include enzyme-linked immunoassays (ELISA), immunoblotting and immunoprecipitation (see Voller, 1978, Diagnostic Horizons, 2:1, Microbiological Associates Quarterly Publication, Walkersville, MD; Voller et al., 1978, J. Clin. Pathol., 31: 507; U.S. Reissue Pat. No. 31,006; UK Patent 2,019,408; Butler, 1981, Methods Enzymol., 73: 482; Maggio, E. (ed.), 1980, Enzyme Immunoassay, CRC Press, Boca Raton, FL) or radioimmunoassays (RIA) (Weintraub, B., Principles of radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March 1986, pp. 1-5, 46-49 and 68-78). For analysing tissues for the presence or absence of a protein produced by a candidate gene according to the present invention, immunohistochemistry techniques may be used. It will be apparent to one skilled in the art that the antibody molecule may have to be labelled to facilitate easy detection of a target protein. Techniques for labelling antibody molecules are well known to those skilled in the art (see Harlow and Lane, 1989, Antibodies, Cold Spring Harbor Laboratory).

## J. Preparation of a Labeled Protein

### 1. Labling of protein

Labeling techniques are useful, according to the invention, for studying the biochemical properties, processing, intracellular transport, secretion and degradation of proteins.

Biosynthetic labeling of proteins produced by candidate genes of the invention is preferably performed with <sup>35</sup>S-methionine due to the high specific activity (>800Ci/mmol) and ease of detection of this amino acid. Another amino acid should be used to label a protein that contains little or no methionine.

According to the following protocol, either suspension cells or adherent cells are labeled with <sup>35</sup>S-methionine. Briefly, cells are washed and incubated for 15 min at 37°C in short-term labeling medium (complete serum-free, methionine free RPMI or DMEM containing 5% (v/v) dialyzed fetal bovine serum) to deplete intracellular pools of methionine. Cells are then incubated in the presence of <sup>35</sup>S-methionine working solution (0.1 to 0.2 mCi/ml in 37°C short-term labeling medium) such that 4ml of <sup>35</sup>S-methionine working solution is added per 2 x 10<sup>7</sup> suspension cells and 2 to 4 ml of <sup>35</sup>S-

methionine working solution is added per 100 mm dish of adherent cells ( $0.5-2 \times 10^7$  cells), for a period of 30 min to 3 hour in a humidified, 37°C, 5% CO<sub>2</sub> incubator. Upon completion of labeling, suspension cells are washed by centrifugation in ice-cold PBS. Following removal of labeling medium, adherent cells are washed with PBS, scraped and collected by centrifugation. Labeled cells are processed and analyzed by immunoaffinity chromatography, immunoprecipitation and one- and two-dimensional gel electrophoresis (Ausubel et al., supra).

If the protein of interest is synthesized at a relatively low rate or is in a steady state, it may be necessary to label cells for an extended period of time. When performing long-term biosynthetic labeling of cells, it is necessary to include unlabeled methionine in the medium to maintain cell viability and to ensure that incorporation of label is maintained during the course of the experiment. According to this method, cells can be labeled in the presence of <sup>35</sup>S-methionine in long term labeling medium (90% methionine free RPMI or DMEM) for up to 16 hours (Ausubel et al., supra).

## 2. *In vitro* Translation

The protein product of the cloned candidate gene of the invention can be produced by the methods of *in vitro* transcription and *in vitro* translation. *In vitro* transcription is performed essentially as described in Section B entitled "Production of a Polynucleotide Sequence" in the absence of a labeled ribonucleoside. The RNA produced by the *in vitro* transcription reaction will be extracted with phenol, ethanol precipitated twice and resuspended in 10ml of TE buffer. *In vitro* translation is performed by adding 1 to 10ml of RNA to an *in vitro* translation kit (e.g. wheat germ or reticulocyte lysate) in the presence of 15mCi [<sup>35</sup>S]methionine, following the directions provided by the manufacturer. A typical reaction is carried out in a 30ml volume at room temperature for 30 to 60 minutes (Ausubel et al., supra).

## K. Production of Cells Expressing a Nucleotide Sequence Comprising a Polymorphism

Mammalian cells expressing a nucleotide sequence comprising a polymorphism are useful, according to the invention for determining the biochemical and functional properties of the protein product of a nucleotide sequence comprising a polymorphism, for analyzing expression of a candidate gene, for large scale production of a protein of interest, for drug screening and for the production of transgenic animals or knockout mice.

Methods of efficiently introducing foreign DNA into mammalian cells are known in the art and include calcium phosphate transfection, DEAE-dextran transfection, electroporation and liposome-

mediated transfection (Ausubel et al., supra).

### Transfection Protocols

#### 1. Calcium-Phosphate Transfection

The method of calcium phosphate transfection involves preparing a precipitate by slowly mixing a HEPES-buffered saline solution with a mixture of calcium chloride and DNA. According to this method, up to 10% of the cells on a dish will incorporate DNA.

Cells to be transfected are split one day prior to transfection so that on the day of transfection cells are well-separated on the plate. a 10 cm dish of cells is fed with 9.0 ml of complete medium approximately 2 to 4 hours before the addition of the precipitate. DNA to be transfected (10-50mg/10-cm plate) is ethanol precipitated, resuspended in 450 ml sterile water and mixed with 50 ml of 2.5 M  $\text{CaCl}_2$ . The DNA/ $\text{CaCl}_2$  solution is added dropwise to a 15-ml conical tube containing 500 ml 2X HeBS (0.283M NaCl, 0.023M HEPES acid, 1.5 mM  $\text{Na}_2\text{HPO}_4$ , pH 7.05). It is preferable to bubble the HeBS solution during the addition of the DNA mixture. After the precipitate has formed for 20 minutes at room temperature, it is added evenly to the cells. The cells are incubated with the precipitate at 37°C in a  $\text{CO}_2$  humidified incubator for 4-16 hours. Following removal of the precipitate, the cells are washed with PBS and fed in complete medium. Glycerol or dimethyl sulfoxide shock can be used to increase the DNA uptake by certain types of cells (Ausubel et al., supra).

#### 2. DEAE-Dextran Transfection

Cells to be transfected are plated at a concentration such that after 3 days of growth they are 30-50% confluent. The DNA to be transfected (approximately 4 mg) is ethanol precipitated, resuspended in 40ml TBS and added slowly while shaking to 80 ml of warm 10 mg/ml DEAE-dextran in TBS. After cells have been washed with PBS and fed with 4 ml of DMEM containing 10% Nu Serum/10cm dish, the DEAE-dextran/DNA mixture is evenly distributed over the entire plate. Cells are incubated with the DNA for approximately 4 hours in a humidified  $\text{CO}_2$  incubator. Following the removal of the DEAE-dextran/DNA mixture, cells are shocked by the addition of 5 ml of 10% DMSO in PBS. After a 1 minute incubation at room temperature, cells are washed with PBS and fed with complete medium (Ausubel et al., supra).

#### 3. Electroporation

Alternatively, DNA can be introduced into cells by the use of high-voltage electric shocks, a technique termed electroporation. Briefly, according to the method of electroporation, cells are

suspended in an appropriate electroporation buffer and placed in an electroporation cuvette. Following the addition of DNA, the cuvette is connected to a power supply and the cells are subjected to a high-voltage electrical pulse of a defined magnitude and length, optimized for the cell type being transfected. After a brief period of recovery, the cells are placed in normal culture medium.

5 A population of cells to be transfected by electroporation is grown to late-log phase in complete medium. Typically stable transfection requires  $5 \times 10^6$  cells, and transient transfection requires  $1-4 \times 10^7$  cells. Cells are harvested by centrifugation for 5 minutes at  $640 \times g$  at  $4^\circ\text{C}$ . The resulting cell pellet is resuspended in half of the original volume of ice-cold electroporation buffer (e.g. PBS without calcium or magnesium, Hepes buffered saline, tissue culture medium without serum, or  
10 phosphate buffered sucrose (272mM sucrose/7 mM  $\text{K}_2\text{HPO}_4$ , pH 7.4/1mM  $\text{MgCl}_2$ )). The choice of an electroporation buffer is dictated by the cell line. Cells are then harvested by centrifugation for 5 minutes at  $640 \times g$  at  $4^\circ\text{C}$ , and resuspended at  $1 \times 10^7/\text{ml}$  in electroporation buffer at  $0^\circ\text{C}$  for stable transfection or at a higher concentration (up to  $8 \times 10^7/\text{ml}$ ) for transient transfection. Aliquots of the cells (0.5 ml) are transferred into the desired number of electroporation cuvettes and placed on ice.

15 DNA is added to the cell suspension in the cuvettes on ice. For stable transfection, DNA (optimally 1-10 mg) should be linearized with a restriction enzyme that cuts at a site in a non-essential region, purified by phenol extraction and ethanol precipitated. Supercoiled DNA (optimally 10 mg) may be used for transient transfection. The DNA/cell suspension is mixed, and incubated on ice for 5 minutes.

20 The cuvette is placed in the holder in the electroporation apparatus (at room temperature) and shocked one or more times at the desired voltage and capacitance settings. An electroporation apparatus useful according to the invention is the Bio-Rad Gene Pulser. The number of shocks and the voltage and capacitance settings will vary depending on the cell type, and should be optimized. The two parameters that are critical for successful electroporation are the maximum voltage for the shock  
25 and the duration of the current pulse.

Following electroporation, the cuvette containing the mixture of cells and DNA is incubated on ice for 10 minutes. The transfected cells are diluted 20-fold in complete culture medium. For stable transfection cells are grown for 48 hours in nonselective medium and then transferred to antibiotic containing medium. For transient transfection, cells are incubated 50-60 hours and then harvested for  
30 the desired transient assay.

## **L. Production of Animals Expressing a Nucleotide Sequence Comprising a Polymorphism**



Transgenic animals expressing a construct comprising a candidate gene containing a polymorphism, according to the invention can be produced by methods well known in the art (reviewed in Reeves et al., supra). Knock out mice wherein a candidate gene according to the invention has been disrupted can be produced by methods well known in the art (reviewed in Moreadith and Radford, 1997, J.Mol. Med., 75:208 and Shastry, 1998, Mol. Cell. Biochem., 181:163). These animals provide useful models for studying the functional consequences of one or more polymorphisms in a gene of interest.

#### **M. Production of a Candidate Gene Library**

The invention provides a method of producing a candidate gene library comprising genes that are potentially associated with the susceptibility to, or pathogenesis of a disease. A candidate gene library is useful for determining the genetic basis of a disease of interest.

Genetic susceptibility to a disease must occur as a result of specific DNA differences relative to non-susceptible individuals. In the case of osteoarthritis, many genes are known which are potentially involved in the susceptibility to, or pathogenesis of the disease. These genes are included in the candidate gene library and the association of these genes with osteoarthritis is determined from population studies according to the invention. Unlike linkage studies wherein a region of the genome that is thought to be involved in a disease is determined, the candidate gene strategy, including association studies, addresses the involvement of a particular gene in a disease. The results of association studies of candidate genes are used to identify genes that should be intensively studied as potential therapeutics or therapeutic targets.

According to the invention, the full range of polymorphic sites within each candidate gene is identified and examined in diseased and normal populations. The frequency of each gene variant (allele) in each population is then compared to the other. If a specific polymorphism under analysis contributes to the disease phenotype, it will be present in the diseased population at a higher frequency than in the normal population. In addition, if the specific polymorphism under analysis does not itself contribute to the disease phenotype but resides elsewhere in, or is near to a gene containing a contributory polymorphism, a significant association may be seen with the polymorphic marker being tested. This is because the two markers are in linkage disequilibrium with each other due to their close proximity.

##### **1. Strategies for Identifying Genes Associated with a Disease**

There are a number of methods known in the art for the identification of genes involved in a

disease. These methods include familial linkage studies followed by positional cloning, differential gene expression studies on tissues, and population-based candidate gene association studies. Although positional cloning has proven to be useful for diseases resulting from a single mutation, this technique is not suitable for identifying genetic linkage in diseases where multiple genetic variants combine to create disease susceptibility. Furthermore, it has been demonstrated that the etiological basis of the majority of diseases comprises more than one gene.

The goal of linkage studies is to determine the approximate position of disease genes by studying related individuals in families. According to linkage strategies, DNA markers that are randomly spaced throughout the genome, but are rarely located within genes, are tested for the frequency of their presence along with the particular disease phenotype. There is approximately a 50% chance of an unlinked gene and marker gene co-localizing. If a particular marker is present at a significantly higher frequency than expected in disease individuals, this indicates that the marker is located in the vicinity of the disease gene. Usually the disease gene is delimited to a large region (containing tens to hundreds of genes). After a disease gene has been grossly mapped, this entire region must be extensively characterized to determine what genes are present in the region. Any gene that is identified according to this method becomes a candidate gene.

Linkage studies have been used successfully to identify the genes responsible for certain genetic diseases originating from mutations in a single gene (monogenic diseases). However, most common human diseases are of polygenic origin wherein changes in multiple genes causes an increased susceptibility to or pathogenesis of a particular disease. Because the DNA changes associated with genes which contribute to polygenic diseases are common in the population, thereby diluting the contribution of a given region of the genome to the disease, it is difficult to perform linkage studies on diseases of polygenic origin.

#### Linkage analysis

A series of genetic crosses is performed in an animal model system of a particular defect that is characteristic of a disease of interest (e.g. osteoarthritis) between individuals having an observable mutant phenotype and normal individuals of a control strain. At least one disease-related loci is used as a marker in these crosses. Alternatively, linkage analysis can be performed using chromosomal markers that do not comprise a disease related locus (described below). If non-random assortment of the mutant trait with a marker locus is observed, and if that non-random assortment is statistically significant (for example, if a Student's t test or ANOVA is applied to the results) the trait is linked to the marker locus.

Similarly, linkage analysis using an existing human or other mammalian pedigree may be performed. Pedigree analysis is a useful technique for identifying genes for which variant alleles may contribute to the risk, onset or progression of a disease in a family containing multiple individuals afflicted with a disease; according to this method, numerous genetic loci from affected and unaffected family members are compared. Non-random assortment of a given genetic marker between affected and unaffected family members relative to the distributions observed for other genetic loci indicates that the marker (for example, a variant isoform of a gene) either contributes to the disease or is in physical proximity to another that does so.

If a non-random assortment of the disease-related phenotype with a marker locus is observed, using either approach, this is indicative of an association between the gene underlying the defect and that locus. Because the strength of any conclusion drawn from linkage analysis is statistically-based, the accuracy of the results is thought to be proportional to the number of crosses or family members and genetic loci analyzed.

#### Positional Cloning

If linkage is confirmed it is preferable to perform a molecular analysis of the region in which the peak of linkage maps. The wide availability of yeast artificial chromosome (YAC) or bacterial artificial chromosome (BAC) libraries facilitates this analysis. A nucleic acid sequence specific for a region encompassing a gene which is determined to occupy a map location of a particular locus of interest is examined, and open reading frames are evaluated to determine their relationship with the observed phenotype. An initial evaluation may be performed with the assistance of a computer program, such as the PathCalling™ (CuraGen) biological pathway discovery platform. All or a subset of the open reading frames present in the region are then cloned (e.g., by PCR) from mutant animals or affected family members and from their healthy counterparts (either control animals or unaffected family members), and the sequences of these open reading frames are compared. If a mutation or other allelic variant is found to be linked to individuals displaying the disease phenotype (in a statistically-significant, non-random manner), it can be concluded that this mutation is associated with a disease phenotype. A nucleic acid fragment containing this gene can be labeled and used as a probe for *in situ* hybridization analysis of fixed chromosomes of the human or other mammal to determine precisely the physical location of the gene. Furthermore, a gene that has been mapped and isolated in this manner may be useful as a candidate target for disease diagnosis and for drug targeting according to the invention (see below).

## 2. Identification of Genes to be Included in Candidate Gene Library

A candidate gene library according to the invention will include i. genes that are involved in known or predicted disease pathways, ii. new genes that are identified by a relevant pattern of specific tissue or cell expression, iii. genes that map to genomic regions of known linkage, and iv. gene sequences (from sequence databases) that are homologs of the above referenced categories of potential candidate genes. The choice of potentially related genes to be selected from a database will depend on the percent identity as calculated by Fast DB and based upon mismatch penalty, gap penalty, gap size penalty and joining penalty. Figure 1 summarized

Based on the physiological changes associated with a disease of interest, predictions can be made regarding a cell or tissue-type that would be expected to express high or low levels of candidate genes associated with a particular disease. For osteoarthritis, it is expected that muscle, adipose, pancreas or liver tissue or tissue comprising insulin secreting pancreatic b-cells, would be useful for identifying candidate genes according to the invention.

Differences in the expression of known and unknown genes in normal and disease tissue can be determined by methods known in the art including Serial Analysis of Gene Expression (SAGE) (Velculescu et al., 1995, Science, 270:484), subtractive hybridization/screening (described below), differential display (Ling and Pardee, 1992, Science, 257:967) high-density microarray expression testing.

The technique of SAGE allows for the rapid, detailed analysis of thousands of transcripts. SAGE depends on the following two principles. First, sufficient information is contained within a short nucleotide sequence (approximately 9-10bp), isolated from a defined location within a transcript, to uniquely identify a transcript. Second, the concatenation of short tags of sequence allows transcripts to be analyzed serially by sequencing multiple tags within a single clone.

The method of SAGE is performed by synthesizing double-stranded cDNA from mRNA, cleaving the resulting cDNA with an anchoring restriction endonuclease that is expected to cleave most transcripts at least one time, and isolating the most 3' region of the cleaved cDNA by binding to streptavidin beads. This protocol allows for the identification of a unique site on a transcript that corresponds to the restriction site located closest to the polyA tail. Replicate samples of the most 3' region of the cDNA are ligated to one of two linker molecules that contain a type IIS restriction site for a tagging enzyme. The cleavage site for Type IIS restriction endonucleases is located at a defined distance up to 20 bp from the asymmetric recognition site. Linkers are designed such that upon cleavage of the ligation product with the tagging enzyme there is release of the linker and an attached short region of cDNA.

Following the creation of blunt ends, the two pools of released tags are ligated to each other and the resulting ligated product is used as a template for PCR amplification in the presence of primers that are specific for each linker. The PCR product is cleaved with the anchoring enzyme and amplification products, comprising two tags linked tail to tail, are isolated, concatenated by ligation, cloned and sequenced (Velescu et al., supra).

Differential display provides a method for separating and cloning individual mRNAs by PCR analysis. According to the method of differential display, oligonucleotide primers are selected wherein one primer is anchored to the polyadenylate tail of a subset of mRNA species and the other primer is short and of an arbitrary sequence such that it anneals at different positions relative to the first primer. The mRNA subpopulations that are identified with these primer pairs are subjected to reverse transcription, amplified and analyzed on a DNA sequencing gel. By using multiple sets of primers, a reproducible pattern of amplified cDNA fragments that demonstrate a requirement for the sequence specificity of either primer can be obtained (Liang and Pardee, supra).

According to the method of high-density microarray expression testing, DNA sequences to be tested for expression are spotted onto a surface, usually at high-density to allow for the testing of many genes. The surface containing the DNA sequences is typically referred to as a 'chip'. The spotted DNA can be either cDNA clones or oligonucleotides. RNA is prepared from the two cells or tissues to be compared. The RNA from one cell/tissue will be labeled red and the RNA from the other cell/tissue will be labeled yellow. Both RNA preparations are hybridized to the DNA array. The ratio of red to yellow is indicative of the relative levels of expression between the two cells/tissues.

### 3. Mapping a candidate gene

Molecular and cytogenetic methods of mapping candidate genes are known in the art and are summarized below. Linkage analysis provides a method for identifying genes mapping to genomic regions of known linkage.

#### Linkage analysis

As described above, linkage analysis may be performed between an unmapped candidate gene and one or more of the disease-related loci or by analyzing the genetic linkage between the candidate gene and chromosomal markers which are not themselves linked to a disease-related locus, according to the same method. For the latter type of analysis it is preferable that the spacing of markers throughout the genome of the test organism is approximately one every cM or less. This spacing will ensure complete coverage of the genome and will facilitate accurate mapping.

Other methods for mapping a candidate gene are provided below.

#### Syntenic similarity

As a result of classical genetic studies and, more recently, multi-laboratory genomic  
5 sequencing collaborations such as the Human Genome Project and Mouse Genome Project, the  
human and mouse genomes have been extensively characterized. It is now known that there is a  
significant degree of co-linearity among human, mice and rats wherein there is conservation relative to  
one another among these several species in the chromosomal map positions of numerous genes and  
groups of genes. Examination of the human and/or mouse chromosomal maps in the regions  
10 comparable to those to which a particular loci of interest maps in the rat will yield candidate genes  
which may be responsible for the physiological changes associated with a disease of interest. The  
methods of radiation hybrid mapping or fluorescence *in situ* hybridization at low stringency to rat  
chromosomes using labeled fragments derived from the human or mouse genes can be used to  
confirm that genes present in these regions of the human and/or mouse are present in the regions of  
15 interest in the rat.

Radiation hybrid (RH) mapping is a somatic cell hybrid technique that was developed to  
create high resolution, contiguous maps of mammalian chromosomes. The method is useful for  
ordering DNA markers spanning millions of base pairs of DNA at a resolution not easily obtained by  
other mapping methods (Cox et al., 1990, Science, 250: 245; Burmeister et al., 1991, Genomics, 9:19;  
20 Warrington et al., 1992, Genomics, 13: 803; Abel et al., 1993, Genomics, 17:632). Radiation hybrid  
mapping facilitates the mapping of non-polymorphic DNA markers that cannot be used for meiotic  
mapping.

According to the method of radiation hybrid mapping a lethal dose of X-irradiation is used to  
fragment the chromosomes of the donor cell line. Chromosome fragments from the donor cell line are  
25 then retained, in a non-selective manner, following cell fusion with a recipient cell line. The resulting  
hybrid clones are then analyzed for the presence or absence of specific donor chromosome markers.  
It is expected that markers that are further apart on a chromosome are more likely to be broken apart  
by radiation and to segregate independently in the RH cells than markers that are closer together. By  
performing a statistical analysis of the co-segregation of various loci in hybrid clones, it is possible to  
30 construct a map that provides information regarding the relative order and distance of markers (Cox et  
al., 1990, supra; Warrington et al., 1991, Genomics, 11: 701; Ceccherini et al., 1992, Proc. Natl. Acad.  
Sci. USA, 89: 104).

### Subtractive screening

In view of the observation that only a subset of an organism's genes are expressed in a given tissue, there is a high probability that transcripts which differ in expression between cells of the same tissue in a mutant and control animal are responsible for the observed mutant phenotype.

5 According to the method of subtractive cloning, mRNA is isolated from a tissue of choice, wherein the tissue is obtained from two distinct organisms and wherein one organism displays a mutant phenotype with regard to a particular trait while the other is normal in that respect. Methods well known in the art are used to prepare cDNA from the mRNA derived from the organism. The mRNA template is then degraded, either by hydrolysis under alkaline conditions or by RNAase H-  
10 mediated cleavage, and the cDNA is returned to a buffer in which mRNA is stable, and mixed with a molar excess of mRNA prepared from the second organism under conditions of stringent hybridization. The mixture is then passed over a hydroxyapatite column, which binds double-stranded nucleic acids but allows single stranded nucleic acid molecules to pass through. Reverse transcripts derived from the first sample which do not hybridize to mRNA molecules derived from the second  
15 organism (in other words, reverse transcripts specific to the first tissue sample) are present in the flow-through fraction and are cloned into a vector to create a subtraction library. The reciprocal experiment (in which the cDNA is derived from the second mRNA preparation) is also carried out to create a complete set of transcripts specific to the tissue samples derived from the two organisms.

This procedure will provide transcripts that can be labeled and used as probes in *in situ*  
20 hybridization analysis of immobilized chromosomes. The method of subtractive screening therefore, yields both cloned genes as well as reagents useful for determining if the cloned genes co-localize with a loci of interest. If a particular gene is found to co-localize to a loci of interest, the genes may be analyzed functionally (e.g., in a phenotypic rescue experiment, as described below or by the phenotypic assays described in Section F entitled "Identification and Characterization of  
25 Polymorphisms") Ultimately, these genes may be used as targets for drugs or disease diagnostic methods, or even as therapeutic nucleic acids.

### Mutagenic transposon mapping

The selection of insertional events that lie within genes (e.g., within coding or regulatory  
30 sequences) is facilitated by the use of entrapment vectors, first described in bacteria (Casadaban and Cohen, 1979, Proc. Natl. Acad. Sci. U.S.A., 76: 4530; Casadaban et al., 1980, J Bacteriol, 143: 971). By employing animal models, entrapment vectors can be introduced into pluripotent ES cells in culture (for example, using electroporation or a retrovirus) and then passed into the germline via chimeras

(Gossler et al., 1989, Science, 244: 463; Skames, 1990, Biotechnology, 8:827). Alternatively, transgenic animals containing entrapment vectors may be generated by standard oocyte injection protocols.

These methods result in DNA integrations that are highly mutagenic because they interrupt the endogenous coding sequence. It is estimated that the frequency of obtaining a mutation in some gene of any in the genome using a promoter or gene trap is about 45%. For a detailed description of retroviral insertion mutagenesis see Methods Enzymol., vol. 225, 1990. Genes which are expressed in a tissue of interest and for which a biochemical assay of a particular activity have been developed in animal models are most useful according to this method. Promoter or gene trap vectors often contain a reporter gene, e.g., *lacZ*, *Cat* or *green fluorescent protein (Gfp)* that lacks its own upstream promoter and/or splice acceptor sequence. That is, promoter gene traps contain a reporter gene with a splice site but no promoter. If the vector integrates within a gene and is spliced into the gene product, then the reporter gene will be expressed. Enhancer traps contain a reporter gene and have a minimal promoter which requires the activity of an enhancer in order to function. If the vector integrates near an enhancer (whether in a gene or not), then the reporter gene will be expressed. Activation of the reporter gene can only occur when the vector is integrated within an active host gene and generates a fusion transcript with the host gene. The activity of a reporter gene provides an easy assay for determining if a vector has been integrated into an expressed gene. Methods for detecting reporter gene activity in transfected cells or tissues of a transgenic animal are well known in the art.

The mutagenic vector may be mapped using standard cytogenetic techniques, such as *in situ* hybridization, wherein a labeled fragment comprising vector-specific sequence is used as a probe. Co-localization of the probe with a particular locus of interest indicates that the associated gene is a suitable candidate and should be subjected to further analysis. A gene that has been identified in this manner can be cloned as described.

#### N. Diagnostic Indicators, Screens and Disease Symptoms

In another embodiment of the invention, there is provided a method of diagnosing or determining susceptibility of a subject to joint space narrowing and/or osteophyte development and/or joint pain. This method involves analyzing the genetic material of a subject to determine which allele(s) of a gene is/are present. The method may include determining whether one or more particular alleles are present, or which combination of alleles (i.e. a haplotype) is present. The method may also include determining whether subjects are homozygous or heterozygous for a particular allele or haplotype.

In a preferred embodiment, the method comprises determining which allele of one or more



polymorphisms of the invention is/are present. In particular, the method may include determining the presence of a polymorphism of a gene which in combination with polymorphisms defined herein or other polymorphisms may define a risk haplotype. The polynucleotides sequences for these particular alleles may be used for diagnostic purposes. The polynucleotides which may be used  
5 include oligonucleotides, complementary RNA and DNA molecules and PNAs. The polynucleotides may be used to determine whether subjects are homozygous or heterozygous for a particular allele or haplotype making them susceptible to joint space narrowing and/or osteophyte development and/or joint pain, and hence, osteoarthritis.

In one aspect, hybridization with a PCR probe which is capable of detecting a particular  
10 polymorphism may be used to identify nucleic acid sequences of particular alleles or haplotype. These probes must be specific to these particular alleles and the stringency of the hybridization or amplification must be such that the probe identifies only this particular allele.

Means for producing specific hybridization probes for these polynucleotides of particular alleles include the cloning of these polynucleotide sequences into vectors for the production of  
15 mRNA probes is well known to one skilled in the art. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as  $^{32}\text{P}$  or  $^{35}\text{S}$ , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via  
20 avidin/biotin coupling systems, and the like.

Polynucleotides of particular alleles or haplotype may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect susceptibility to joint space narrowing and/or osteophyte development and/or joint pain. Such  
25 qualitative methods are well known in the art.

In a particular embodiment, polynucleotides of particular alleles or haplotype may be used in assays that detect susceptibility to joint space narrowing and/or osteophyte development and/or joint pain, particularly those mentioned above. Polynucleotides complementary to sequences of a particular allele or haplotype may be labeled by standard methods and added to a fluid or tissue  
30 sample from a patient under conditions suitable for the formation of hybridization complexes. After

a suitable incubation period, the sample is washed and it is determined if there is a signal. If a signal is found, then the presence of the polynucleotide of a particular allele, alleles or haplotype in the sample indicates the susceptibility to joint space narrowing and/or osteophyte development and/or joint pain, and hence, osteoarthritis. Such assays may also be used to determine the particular  
5 therapeutic treatment regimen for an individual patient.

With respect to osteoarthritis, the presence of a particular polymorphism or polymorphisms in a tissue sample from an individual may indicate a predisposition for joint space narrowing and/or osteophyte development and/or joint pain, or may provide a means for detecting osteoarthritis prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow  
10 health professionals to employ preventative measures or aggressive treatment earlier, thereby preventing the development or further progression of osteoarthritis.

Additional diagnostic uses for oligonucleotides designed from the polynucleotide sequences of a particular allele or haplotype may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced *in vitro*. Oligomers will contain a fragment of a  
15 polynucleotide a particular allele, alleles or haplotype or a fragment of a polynucleotide complementary to the polynucleotide a particular allele, alleles or haplotype, and will be employed under optimized conditions for identification of a specific polymorphism, polymorphisms or haplotype. Oligomers may also be employed under very stringent conditions for detection of these particular DNA or RNA sequences.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotides described herein may be used as elements on a microarray. The microarray can be used in transcript imaging techniques to detect a particular polymorphism, polymorphisms or haplotype simultaneously as described below. In particular, this information may be used to develop a pharmacogenomic profile of a patient in order to select the most appropriate and effective  
25 treatment regimen for that patient. For example, therapeutic agents which are highly effective and display the fewest side effects may be selected for a patient based on his/her pharmacogenomic profile.

Microarrays may be prepared, used, and analyzed using methods known in the art (Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl.  
30 Acad. Sci. USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116;

Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662). Various types of microarrays are well known and thoroughly described in Schena, M., ed. (1999; DNA Microarrays: A Practical Approach, Oxford University Press, London).

5 In another embodiment, a method involves the use of antibodies in diagnosing or determining the susceptibility to joint space narrowing and/or osteophyte development and/or joint pain. The antibodies would specifically bind to an epitope of a particular allele or form of the protein and may be used to determine susceptibility to joint space narrowing and/or osteophyte development and/or joint pain, and hence, osteoarthritis. Antibodies useful for diagnostic purposes may be prepared in  
10 the same manner as described above. Diagnostic assays for determining susceptibility to joint space narrowing and/or osteophyte development and/or joint pain include methods which utilize the antibody and a label to detect a particular allele or form of the protein in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter  
15 molecules are known in the art and may be used.

A variety of protocols for measuring a particular allele or form of the protein, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing susceptibility to joint space narrowing and/or osteophyte development and/or joint pain.

## 20 O. Preparation of a Human Sample

The presence of an allelic form of a gene containing a sequence variation, according to the invention, can be detected by testing any tissue of a human subject. Human samples that are useful according to the invention include tissue or fluid samples containing a polynucleotide or polypeptide of interest, include but are not limited to plasma, serum, spinal fluid, lymph fluid, urine, stool, external  
25 secretions of the skin, respiratory, intestinal and genitoruinary tracts, saliva, blood cells, tumors, organs, tissue and samples of *in vitro* cell culture constituents. Genomic DNA, cDNA or RNA can be prepared from the human sample according to the methods described above.

## P. Methods of Use

### 30 1. Nucleic Acid Diagnosis and Diagnostic Kits

In order to detect the presence of an allele of a gene predisposing an individual to

osteoarthritis, a biological sample such as blood is prepared and analyzed for the presence or absence of susceptibility alleles of a gene containing a polymorphism, according to the invention. Results of these tests and interpretive information will be returned to the health care provider for communication to the tested individual. Such diagnoses may be performed by diagnostic laboratories, or, alternatively, diagnostic kits are manufactured and sold to health care providers or to private individuals for self-diagnosis.

Initially, the screening method will involve amplification of the relevant gene sequences. In another preferred embodiment of the invention, the screening method involves a non-PCR based strategy. Such non-PCR based screening methods include Southern blot analysis to detect the presence of a variant form of a gene in a sample comprising total genomic DNA from the individual being tested. Alternatively, northern blot analysis can be used to detect an aberrant mRNA encoded by a gene, that exhibits altered stability or is the result of alternative splicing in a sample comprising RNA from an individual being tested. The methods of S1 nuclease analysis, RNase protection and primer extension can also be used to determine both the endpoint and the amount of a gene specific mRNA (Ausubel et al., supra). Both PCR and non-PCR based screening strategies can detect target sequences with a high level of sensitivity.

The preferred method, according to the invention, is target amplification. According to this method, the target nucleic acid sequence is amplified with polymerases. One particularly preferred method using polymerase-driven amplification is PCR (described above). The polymerase chain reaction and other polymerase-driven amplification assays can achieve over a million-fold increase in copy number through the use of polymerase-driven amplification cycles. PCR primers useful for target amplification according to the invention, will be designed to amplify a region of DNA containing one or more polymorphisms. Allele specific primers (comprising one or more polymorphisms) are also useful for detecting gene sequence variations by PCR methodologies according to the invention. The absence of a particular polymorphism will be indicated by the absence of an amplified product when the amplification step is carried out in the presence of allele specific primers. Once amplified, the resulting nucleic acid can be sequenced and the specific sequence of the test DNA will be compared with the wild type sequence by using the computer programs described in Section F entitled "Identification and Characterization of Polymorphisms". Alternatively, the amplified product will be analyzed by Southern blot assay with nucleic acid probes. Nucleic acid probes, useful according to the invention, will be specifically hybridizable to a mutant form of a gene but not to the wild type gene due to the presence of one or more polymorphisms.

When a probe comprising the target sequence, according to the invention, is used to detect the

presence of the target sequences via non PCR-based strategies, (for example, in screening for osteoarthritis susceptibility), the biological sample to be analyzed, such as blood or serum, may be treated, if desired, to extract the nucleic acids (as described above). The sample nucleic acids (isolated from a biological sample or amplified by PCR) may be prepared in various ways to facilitate detection of the target sequence; e.g. denaturation, restriction digestion, electrophoresis or dot blotting. Preferably, the targeted region of the nucleic acids being analyzed are at least partially single-stranded to form hybrids with the targeting sequence of the probe. If the sequence is naturally single-stranded, denaturation will not be required. However, if the sequence is double-stranded, the sequence will probably need to be denatured. Denaturation can be carried out by various techniques known in the art.

To detect the presence of a sequence variation in a gene, according to the invention, analyte nucleic acid and probe will be incubated under conditions which promote stable hybrid formation of the target sequence in the probe with the putative targeted sequence in the sample DNA. If the region of the probe which is used to bind to the analyte is designed to be completely complementary to the targeted region, high stringency conditions are desirable in order to prevent false positives. However, conditions of high stringency will be used only if the probes are complementary to regions of the chromosome which are unique in the genome. The stringency of hybridization is determined by a number of factors (described above). Detection, if any, of the resulting hybrid is usually accomplished by the use of labeled probes. Alternatively, the probe may be unlabeled, but may be detectable by specific binding with a ligand which is labeled, either directly or indirectly. Suitable labels, and methods for labeling probes and ligand are known in the art, and are described in Section C entitled "Production of a Nucleic Acid Probe".

Accordingly, the foregoing screening method may be modified to identify individuals having a gene containing a neutral polymorphism not associated with osteoarthritis, by preferably amplifying DNA fragments of a gene derived from a particular individual. The amplified DNA fragments are sequenced and the sequence is compared to the consensus gene sequence containing neutral polymorphisms. At this time, differences between the individual's coding sequence for a gene and a consensus sequence for the same gene are determined wherein the presence of any neutral polymorphisms and the absence of a polymorphisms not previously identified as neutral polymorphisms can be correlated with an absence of increased genetic susceptibility to osteoarthritis resulting from a mutation in a gene coding sequence.

In another embodiment of the invention, detection of a polymorphism will be performed by detecting loss of a restriction enzyme recognition site due to the presence of one or more

polymorphisms. According to this embodiment, a polymorphism will be detected with a polynucleotide probe that is capable of detecting a restriction enzyme fragment containing the polymorphism, wherein the fragment is of a size that can be easily separated on an agarose gel and visualized by Southern blot analysis. A polynucleotide probe according to this embodiment of the invention can be specific for a sequence within the candidate gene or outside of the candidate gene.

It is also contemplated within the scope of this invention that the nucleic acid probe assays of this invention will employ a mixture of nucleic acid probes capable of detecting a gene. Thus, in one example to detect the presence of a gene in a test sample, more than one probe complementary to a gene is employed and in particular the number of different probes is alternatively 2, 3, or 5 different nucleic acid probe sequences. In another example, to detect the presence of mutations in the gene sequence in a patient, more than one probe complementary to a gene is employed wherein the probe mixture includes probes capable of binding to the allele-specific mutations identified in populations of patients with alterations in a gene. In this embodiment, any number of probes can be used, and will preferably include probes corresponding to the major gene mutations identified as predisposing an individual to osteoarthritis.

Northern blot analysis, S1 nuclease analysis, RNase protection and primer extension (Ausubel et al., supra) are also methods according to the invention for detecting changes in mRNA resulting from the presence of one or more polymorphisms in the sequence of a gene.

Additionally, of the methods of genotyping described in Section F entitled "Identification and Characterization of Polymorphisms" can be used for diagnostics according to the invention.

## 2. Peptide Diagnosis and Diagnostic Kits

Osteoarthritis can also be detected on the basis of an alteration of the wild-type polypeptide. Such alterations can be determined by sequence analysis in accordance with conventional techniques. More preferably, antibodies (polyclonal or monoclonal) are used to detect differences in, or the absence of peptides derived from a gene of interest. The antibodies may be prepared as described above in Section I entitled "Preparation of Antibodies". Preferably, antibodies will immunoprecipitate the protein product of a gene from solution as well as react with the protein product of a gene on Western or immunoblots of polyacrylamide gels. Antibodies useful according to the invention will also detect the protein product of a gene in paraffin or frozen tissue sections, using immunocytochemical techniques.

Preferred embodiments relating to methods for detecting wild type or mutant forms of the protein product of a gene include enzyme linked immunosorbent assays (ELISA), radioimmunoassay

(RIA), immunoradiometric assays (IRMA) and immunoenzymatic assays (IEMA), including sandwich assays using monoclonal and/or polyclonal antibodies. Exemplary sandwich assays are described by David et al. In U.S. Pat. Nos. 4,376,110 and 4,486,530, hereby incorporated by reference.

### 3. Drug Screening

This invention is particularly useful for screening therapeutic compounds by using the mutant gene or protein product or binding fragment of the gene in any of a variety of drug screening techniques.

The protein product or fragment of a gene employed in such a test may either be free in solution, affixed to a solid support, expressed on the surface of a cell, or located intracellularly. One method of drug screening utilizes eukaryotic or procaryotic host cells which are stably transformed with a recombinant polynucleotide expressing the polypeptide or fragment, preferably in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. In particular, these cells can be used to measure formation of a complex comprising the protein product or fragment of a gene and the agent being tested. Alternatively, these cells can be used to determine if the formation of a complex between the protein product or fragment of a gene and a known ligand is interfered with by an agent being tested.

Thus, the present invention discloses methods useful for drug screening wherein such methods comprise contacting a candidate drug with a polypeptide or fragment derived from a gene and assaying (i) for the presence of a complex between the drug and the polypeptide derived or fragment derived from a gene, or (ii) for the presence of a complex between the polypeptide or fragment derived from a gene and a ligand, by methods well known in the art. Preferably, the polypeptide or fragment derived from a gene is labeled for use in competitive binding assays. Methods for producing a labeled protein by *in vitro* translation are described in Section J entitled "Preparation of a Labeled Protein". Free polypeptide or fragment will be separated from that present in a protein:protein complex, and the amount of free (i.e., uncomplexed) label will be used as a measure of the binding of the test drug to the polypeptide or the ability of the test drug to interfere with protein:ligand binding.

Another method of drug screening allows for high throughput screening for compounds exhibiting suitable binding affinity to the polypeptides and is described in detail in Geysen, WO 84/03564. According to this method, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or another suitable surface. The peptide test compounds are reacted with the polypeptides or peptide fragments derived from a gene, and washed. Bound polypeptide is then detected by methods well known in the art.

Purified protein can be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies to the polypeptide can be used to capture the polypeptide or peptide fragment of interest and immobilize it on the solid support.

Competitive drug screening assays in which neutralizing antibodies capable of specifically binding the polypeptide of interest compete with a test compound for binding to the polypeptide or fragments thereof of interest are also useful according to the invention. According to this method, antibodies can be used to detect the presence of any test peptide which shares one or more antigenic determinants with the polypeptide of interest.

An additional technique for drug screening involves the use of host eukaryotic cell lines or cells (such as described above) which have a gene that produces a defective protein. According to this method, the host cell lines or cells are grown in the presence of a test drug compound. The rate of growth of the host cells is measured to determine if the compound is capable of regulating the growth of cells expressing a nonfunctional protein product of a gene. Alternatively, the ability of the test compound to restore the function of the mutant gene protein can be measured by using an appropriate *in vitro* assay for function of the protein product of a gene. Suitable *in vitro* functional assays are described in Section F entitled "Identification and Characterization of Polymorphisms". If the host cell lines or cells express a protein product of a gene that exhibits an aberrant pattern of cellular localization, the ability of the test compound to alter the cellular localization of the protein will be determined. Changes in the cellular localization of a protein of interest will be detected by performing cellular fractionation studies with biosynthetically labeled cells. Alternatively, the cellular localization of a protein of interest can be determined by immunocytochemical methods well known in the art.

A method of drug screening may involve the use of host eukaryotic cell lines or cells (described above) which have an altered gene that demonstrates an aberrant pattern of expression. By aberrant pattern of expression is meant the level of expression is either abnormally high or low, or the temporal pattern of expression is different from that of the wild type gene. The ability of a test drug to alter the expression of a mutant form of a gene can be measured by Northern blot analysis, S1 nuclease analysis, primer extension or RNase protection assays. Alternatively, if a mutant form of a gene contains an polymorphisms in the promoter region of a gene, cells can be engineered to express a reporter construct comprising a mutant gene promoter driving expression of a reporter gene (e.g. CAT, luciferase, green fluorescent protein). These cells can be grown in the presence of a test compound and the ability of a test compound to alter the level of activity of the mutant gene promoter can be determined by standard assays for each reporter gene which are well known in the art.



### Candidate Drugs

A "candidate drug" as used herein, is any compound with a potential to modulate a phenotype associated with a particular disease according to the invention.

5 A candidate drug is tested in a concentration range that depends upon the molecular weight of the drug and the type of assay. For example, for inhibition of protein/protein complex formation, small molecules (as defined below) may be tested in a concentration range of 1 pg - 100 mg/ml, preferably at about 100 pg - 10 ng/ml; large molecules, e.g., peptides, may be tested in the range of 10 ng - 100 mg/ml, preferably 100 ng - 10 mg/ml.

10 Candidate drug compounds from large libraries of synthetic or natural compounds can be screened. Numerous means are currently used for random and directed synthesis of saccharide, peptide, and nucleic acid based compounds. Synthetic compound libraries are commercially available from a number of companies including Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, NJ), Brandon Associates (Merrimack, NH), and Microsource (New Milford, CT). A rare chemical library is available from Aldrich (Milwaukee, WI). Combinatorial libraries are available and  
15 can be prepared. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available from e.g., Pan Laboratories (Bothell, WA) or MycoSearch (NC), or are readily produceable by methods well known in the art. Additionally, natural and synthetically produced libraries and compounds are readily modified through conventional chemical, physical, and biochemical means.

20 Useful compounds may be found within numerous chemical classes, though typically they are organic compounds, and preferably small organic compounds. Small organic compounds have a molecular weight of more than 50 yet less than about 2,500 daltons, preferably less than about 750 daltons, more preferably less than about 350 daltons. Exemplary classes include heterocycles, peptides, saccharides, steroids, and the like. The compounds may be modified to enhance efficacy,  
25 stability, pharmaceutical compatibility, and the like. Structural identification of an agent may be used to identify, generate, or screen additional agents. For example, where peptide agents are identified, they may be modified in a variety of ways to enhance their stability, such as using an unnatural amino acid, such as a D-amino acid, particularly D-alanine, by functionalizing the amino or carboxylic terminus, e.g. for the amino group, acylation or alkylation, and for the carboxyl group, esterification or  
30 amidification, or the like.

### Determination of Activity of a Drug

A candidate drug, assayed according to the invention as described above, is determined to be

effective if its use results in a change of about 10% of a phenotype associated with a disease according to the invention.

The level of modulation by a candidate modulator of a phenotype associated with a disease according to the invention, may be quantified using any acceptable limits, for example, via the following formula, which describes detections performed with a radioactively labeled probe (e.g., a radiolabeled antibody in an immunobinding experiment or a radiolabeled nucleic acid probe in a Northern hybridization).

$$\text{Percent Modulation} = \frac{(\text{CPM}_{\text{Control}} - \text{CPM}_{\text{Sample}})}{(\text{CPM}_{\text{Control}})} \times 100$$

where  $\text{CPM}_{\text{Control}}$  is the average of the cpm in antibody/ligand complexes or on Northern blots resulting from assays that lack the candidate modulator (in other words, untreated controls), and  $\text{CPM}_{\text{Sample}}$  is the cpm in antibody/ligand complexes or on Northern blots resulting from assays containing the candidate modulator. A similar calculation is performed where the assay comprises use of a labeling system or system of measuring enzymatic activity in which there is a linear relationship between the amount of label detected and the amount of protein or nucleic acid being represented per unit of label or the amount of protein or nucleic acid represented by a unit of enzymatic activity.

#### 4. Rational Drug Design

Rational drug design is useful for producing either structural analogs of biologically active polypeptides of interest or small molecules with which polypeptides of interest interact (e.g., agonists, antagonists, inhibitors) in order to design drugs which are, for example, more active or stable forms of the polypeptide, or which enhance or interfere with the function of a polypeptide *in vivo*. See, e.g., Hodgson, 1991, *BioTechnology*, 9:19. According to one method of rational drug design, the three-dimensional structure of a protein of interest (e.g., the polypeptide product of the gene) or, or the complex comprising the protein product of a gene in association with its ligand, is determined by x-ray crystallography, by computer modeling or most typically, by a combination of approaches.

Alternatively, useful information regarding the structure of a polypeptide may be obtained by modeling based on the structure of homologous proteins. Rational drug design has been used successfully in the development of HIV protease inhibitors (Erickson et al., 1990, *Science*, 249: 527).

Rational drug design may also involve the analysis of peptides derived from the protein

product of a gene by an alanine scan (Wells, 1991, Methods in Enzymol., 202: 390). According to this method, each of the amino acid residues of the peptide is sequentially replaced by alanine, and the effect of this amino acid substitution on the peptide's activity is determined. This technique can be used to determine the functionally relevant regions of the peptide.

5 Another experimental approach to rational drug design will involve the isolation of a target-specific antibody (selected by a functional assay) and the determination of the crystal structure of this antibody. Theoretically, this approach will yield a pharmacore upon which subsequent drug design can be based. Alternatively, if anti-idiotypic antibodies (anti-ids) specific for a functional, pharmacologically active antibody are generated, there is no need to determine the crystallographic  
10 structure of the target-specific antibody. It is expected that the binding site of the anti-ids will be an analog of the original receptor. The anti-id could then be used to identify and isolate potentially therapeutic peptides from banks of chemically or biologically produced banks of peptides. These selected peptides would then function as pharmacores.

According to these methods it may be possible to design drugs which demonstrate increased  
15 activity or stability of the protein product of a gene or which function as inhibitors, agonists, antagonists, etc. of the activity of a protein product of a gene. The availability of cloned gene sequences, including polymorphisms, ensures that sufficient amounts of the polypeptide product of a gene are available to facilitate analytical studies such as x-ray crystallography. Furthermore, the knowledge of the sequence of the protein product of a gene provided herein will guide those using  
20 computer modeling techniques in place of, or in addition to x-ray crystallography.

## 5. Gene Therapy

The present invention also provides a method of supplying wild-type gene function to a cell which carries a mutant allele of a gene. By replacing a mutant gene with a wild type gene, it may be  
25 possible to reverse the symptoms of osteoarthritis in the recipient cells. a full length version of the wild-type gene, or a fragment of the gene, may be introduced into the cell in a vector such that the gene remains extrachromosomal and is expressed by the cell from the extrachromosomal location. More preferably, following introduction into the mutant cell, the wild-type gene or gene fragment should recombine with the endogenous mutant gene X already present in the cell. Such recombination  
30 requires a double recombination event which results in the correction of the gene mutation. Vectors for introduction of genes both for recombination and for extrachromosomal maintenance are known in the art, and any suitable vector may be used. Methods for introducing DNA into cells such as electroporation, calcium phosphate coprecipitation and lipofection are known in the art (described

above). Cells transformed with the wild-type gene can be used as model systems to study changes in the intensity of symptoms associated with osteoarthritis and drug treatments which promote such changes.

As generally discussed above, a gene or a fragment thereof, where applicable, may be used in gene therapy methods in order to increase the amount of the expression products of such genes in cells of patients with osteoarthritis. It may also be useful to increase the level of expression of a gene even in those cells in which the mutant gene is expressed at a "normal" level, but the gene product is not fully functional.

It other embodiments of the invention it may be useful to increase the amount of the expression products of a mutant form of a gene in a cell that expresses the wild type protein. Gene therapy can be carried out according to generally accepted methods, for example, as described by Friedman, 1991, In *Therapy for Genetic Diseases*; T. Friedman ed., Oxford University Press, pp. 105-121). Initially, the appropriate cells from a patient with osteoarthritis would be analyzed by the diagnostic methods described above, to determine the level of production of a polypeptide from a gene and the activity of a polypeptide product of a gene. A virus or plasmid vector (see further details below), comprising a copy of a gene and suitable expression control elements, and capable of replicating inside the cells, will be prepared. Suitable vectors are known and are disclosed in U.S. Pat. No. 5,252,479 and PCT published application WO 93/07282. The vector will be injected into the patient, either locally at an appropriate site according to the invention or systemically.

Gene transfer systems known in the art may be useful in the practice of the gene therapy methods of the present invention. These include viral and nonviral transfer methods. a number of viruses have been used as gene transfer vectors, including papovaviruses, e.g., 5V40 (Madzak et al., 1992, *J Gen Virol.*, 73:1533), adenovirus (Berkner, 1992, *Curr. Top. Microbiol. Immunol.*, 158:39; Berkner et al., 1988, *BioTechniques*, 6:616; Gorziglia and Kapikian, 1992, *J Virol.*, 66:4407; Quantin et al., 1992, *Proc. Natl. Acad. Sci. USA*, 89:2581; Rosenfeld et al., 1992, *Cell*, 68:143 ; Wilkinson et al., 1992, *Nucleic Acids Res.* 20:2233; Stratford-Perricaudet et al., 1990, *Hum. Gene Ther.*, 1:241), vaccinia virus (Moss, 1992, *Curr. Top. Microbiol. Immunol.*, 158:25) adeno-associated virus (Muzyczka, 1992, *Curr. Top. Microbiol. Immunol.*, 158:97; Ohi et al., 1990, *Gene*, 89:279), herpesviruses including HSV and EBV (Margolskee, 1992, *Curr. Top. Microbiol. Immunol.*, 158:67, Johnson et al., 1992, *J. Virol.*, 66:2952; Fink et al., 1992, *Hum. Gene Ther.*, 3:11; Breakfield and Geller, 1987, *Mol. Neurobiol.*, 1:337; Freese et al., 1990, *Biochem. Pharmacol.*, 40: 2189), and retroviruses of avian (Brandyopadhyay and Termin, 1984, *Mol. Cell. Biol.*, 4:749; Petropoulos et al., 1992, *J. Virol.*, 66:3391), marine (Miller, 1992, *Curr. Top. Microbiol. Immunol.*, 158:1; Miller et al., 1985, *Mol. Cell.*

Biol., 5:431; Sorge et al., 1984, Mol. Cell. Biol., 4:1730; Mann and Baltimore, 1985, J. Virol., 54:401; Miller et al., 1988, J. Virol., 62:4337), and human origin (Shimada et al., 1991, J. Clin. Invest., 88:1043); Helseith et al., 1990, J. Virol., 64:24 16; Page et al., 1990, J. Virol., 64: 5370; Buchschacher and Panganiban, 1992, J. Virol., 66:2731). Most human gene therapy protocols have been based on  
 5 disabled murine retroviruses.

Nonviral gene transfer methods known in the art include chemical techniques such as calcium phosphate coprecipitation (Graham and van der Eb, 1973, Virology, 52:456; Pellicer et al., 1980, Science, 209:1414); mechanical techniques, for example microinjection (Anderson et al., 1980, Proc. Natl. Acad. Sci. USA, 77: 5399; Gordon et al., 1980, Proc. Natl. Acad. Sci. USA, 77: 7380; Brinster  
 10 et al., 1981, Cell, 27:223; Constantini and Lacy, 1981, Nature, 294:92); membrane fusion-mediated transfer via liposomes (Felgner et al., 1987, Proc. Natl. Acad. Sci. USA, 84:7413; Wang and Huang, 1989, Biochemistry, 28:9508; Kaneda et al. 1989, J. Biol. Chem., 264:12126; Stewart et al., 1992, Hum. Gen. Ther., 3:267; Nabel et al., 1990, Science, 249:1285; Lim et al., 1992, Circulation, 83:2007); and direct DNA uptake and receptor-mediated DNA transfer (Wolff et al., 1990, Science, 247:1465;  
 15 Wu et al., 1991, J. Biol. Chem., 266:14338; Zenke et al., 1990, Proc. Natl. Acad. Sci. USA, 87:3655; Wu et al., 1989b, J. Biol. Chem., 264:16985; Wolff et al., 1991, BioTechniques, 11:474; Wagner et al., 1990, Proc. Natl. Acad. Sci. USA, 87:3410; Wagner et al., 1991, Proc. Natl. Acad. Sci. USA, 88:4255; Cotten et al., 1990, Proc. Natl. Acad. Sci. USA, 87:4033; Curiel et al., 1991a, Proc. Natl. Acad. Sci. USA, 88:8850; Curiel et al., 1991b, Hum. Gene Ther., 3:147.

20 In an approach which combines biological and physical gene transfer methods, plasmid DNA of any size is combined with a polylysine-conjugated antibody specific to the adenovirus hexon protein, and the resulting complex is bound to an adenovirus vector. The trimolecular complex is then used to infect cells. The adenovirus vector permits efficient binding, internalization, and degradation of the endosome before the coupled DNA is damaged.

25 Liposome/DNA complexes have been shown to be capable of mediating direct *in vivo* gene transfer. While in standard liposome preparations the gene transfer process is nonspecific, localized *in vivo* uptake and expression have been reported in tumor deposits, for example, following direct *in situ* administration (Nabel, 1992, Hum. Gen. Ther., 3:399).

Gene transfer techniques which target DNA directly to an appropriate tissue, e.g., a tissue  
 30 that normally expresses the protein product of the candidate gene of the invention, is preferred. Receptor-mediated gene transfer, for example, is accomplished by the conjugation of DNA (usually in the form of covalently closed supercoiled plasmid) to a protein ligand via polylysine. Ligands are chosen on the basis of the presence of the corresponding ligand receptors on the cell surface of the

target cell/tissue type. These ligand-DNA conjugates can be injected directly into the blood if desired and are directed to the target tissue where receptor binding and internalization of the DNA-protein complex occurs. To overcome the problem of intracellular destruction of DNA, coinfection with adenovirus can be included to disrupt endosome function.

5

## 6. Peptide Therapy

Peptides which have gene activity can be supplied to cells which carry mutant or missing alleles of a gene. Alternatively, peptides specific for a mutant form of the protein product of a gene can be supplied to cells carrying a wild type protein. The protein product of a gene can be produced by expression of the cDNA sequence in bacteria, for example, using known expression vectors (as described in Section H entitled "Production of a Mutant Protein"). Alternatively, the protein product of a gene can be extracted from mammalian cells engineered to produce the protein product of a gene of interest. In addition, the techniques of synthetic chemistry can be employed to synthesize the protein product of a gene. Any of the above techniques can provide a preparation of protein product of a gene that is substantially free of other human proteins. This is most readily accomplished by carrying out protein synthesis in a microorganism or *in vitro*.

Active gene molecules can be introduced into cells by microinjection or by the use of liposomes, for example. Alternatively, some active molecules may be taken up by cells, actively or by diffusion. Extracellular application of the protein product of a gene may be sufficient to decrease or reverse the physiological effects of osteoarthritis. Other molecules with the activity of a protein product of a gene (for example, peptides, drugs or organic compounds) may also be used to effect such a reversal. Modified polypeptides having substantially similar function may also be useful for peptide therapy.

## 7. Transformed Hosts

Cells and animals which carry a mutant allele of a gene can be used as model systems to study and test for substances which have potential as therapeutic agents. Following application of a test substance to the cells, the phenotype of the cell will be determined. Any variety of phenotypic changes associated with osteoarthritis can be assessed, including insulin resistance and combined insulin resistance/insulin secretion defect. Assays for each of these traits are known in the art.

Animals useful for testing therapeutic agents can be selected after mutagenesis of whole animals or after treatment of germline cells or zygotes. Such treatments include insertion of mutant alleles of a gene, usually from a second animal species, as well as insertion of disrupted homologous

genes. Alternatively, the endogenous gene of the animals may be disrupted by insertion or deletion mutation or other genetic alterations using conventional techniques (Capecchi, 1989, Science, 244:1288; Valancius and Smithies, 1991, Mol. Cell. Biol., 11:1402; Hasty et al., 1991, Nature, 350:243; Shinkai et al., 1992, Cell, 68:855; Mombaerts et al., 1992, Cell, 68:869; Philpott et al., 1992, Science, 256:1448; Snouwaert et al., 1992, Science, 257:1083; Donehower et al., 1992, Nature, 356:215).

Following the administration of test substances, the physiological changes associated with osteoarthritis will be assessed. If the test substance prevents or suppresses any of these physiological changes, then the test substance will be considered a candidate therapeutic agent for the treatment of osteoarthritis. These animal models provide an extremely important testing vehicle for potential therapeutic products.

#### 8. Use of a Polynucleotide as a Unique Sequence Marker:

Polynucleotides can be used to mark objects or substances for the purposes of later identification. Thus, polynucleotides of the invention are useful for tracking the manufacture and distribution of a large number of diverse substances, including but not limited to: (1) natural resources such as animals, plants, oil, minerals, and water; (2) chemicals such as drugs, solvents, petroleum products, and explosives; (3) commercial by-products including pollutants such as radioactive or other hazardous waste; and (4) articles of manufacture such as guns, typewriters, automobiles and automobile parts. A nucleic acid according to the invention, when used as a marker, thus aids in the determination of product identity and so provides information useful to manufacturers and consumers.

Polynucleotides have the advantage over other marking materials of being readily amplifiable through the use of polymerase chain reaction (PCR) technology. The method of PCR is well known in the art. PCR is performed as described by Mullis & Faloona, 1987, Methods Enzymol., 155:335, herein incorporated by reference. It is the unique sequence of a polynucleotide which renders it useful as a marker, since this sequence, or a characteristic pattern derived from its sequence, confers a property on the polynucleotide which permits it to be tracked.

It is contemplated that a novel polynucleotide sequence of the invention, or fragments or derivatives of it may be used as markers by their attachment to or mixture in objects or substances to be marked. Methods for marking various classes of substances and later detection of the tags in those substances are disclosed in U.S. Patent Nos. 5,451,505, and 5,643,728.

Briefly, the use of a polynucleotide of the invention as a marker may entail combining a polynucleotide with the substance or object to be marked, using methods appropriate to that substance or object; and detecting the marker through amplification of the polynucleotide sequence using PCR technology, followed by either sequence analysis or identification by other means known in the art

(e.g., hybridization assays).

The methods of applying a marker nucleic acid to a substance or object and subsequent detection of that nucleic acid will vary depending upon the nature of the substance or object and the environment to which it will be exposed. For example, inert solids such as paper, many pharmaceutical products, wood, some foodstuffs, etc., can be either processed with the marker nucleic acid, or the nucleic acid may be sprayed onto their surfaces. Chemically active substances, such as foodstuffs with enzymatic activity, polymers with charged groups, or acidic pharmaceuticals may require that a protective composition (e.g., liposomes) be added to the nucleic acid being used as a marker.

In order to mark liquids, the nucleic acid may be mixed directly with the liquid, or, if the chemical nature of the liquid is not compatible with this approach (i.e., nucleic acids are not soluble in the liquid), the nucleic acid may be mixed with a detergent to enhance its solubility. Containerized gases may be marked simply by adding a nucleic acid to the container in dry form, as it will be dispersed throughout the gas as the gas is released.

The amount of nucleic acid to add to a substance as a marker will also vary with the given situation, as will the detection strategy. PCR technology, however, allows the amplification and detection of as little as one molecule from a sample. Other means of detection, such as hybridization assays require that more nucleic acid be recovered from a sample to efficiently detect it. PCR can be combined with a hybridization assay, however, to enhance the sensitivity of the method.

A nucleic acid sequence used as a marker will generally be from 20 to 1,000 bases long, and preferably will be 60 to 1,000 bases long when PCR is to be used to detect the marker.

One example of a substance for which nucleic acid marking is suited is gunpowder. Marked gunpowder may be prepared as follows: 1) add 16 ng of nucleic acid bearing the chosen marker sequence (derived from a polynucleotide of the invention) to 1 ml of distilled water; 2) mix the solution of nucleic acid with 1 g of nitrocellulose-based gunpowder; and 3) dry in air or under vacuum at 85°C.

To recover the marker from gunpowder: 1) wash the gunpowder sample with 1 ml of distilled water; 2) add 50 ml of the wash solution to a standard PCR mix, or, alternatively, place gunpowder flakes directly into a 100 ml PCR mix; and 3) amplify according to standard PCR methods using primers which anneal at opposite ends and on opposite strands of the sequence used as a marker (annealing and extension conditions will depend upon the exact sequences chosen for oligonucleotide primers, and may be adjusted according to methods known in the art).

Another example of a substance which may be marked with a nucleic acid according to the invention is ink. To prepare marked ink sample: 1) if the ink is water insoluble, mix the nucleic acid with detergents as for oil. If the ink is water soluble, add nucleic acid directly to the ink to a



concentration of about 1 to 20 ng per ml. To recover the marker from ink, proceed as for oils and medicines.

In the above examples, the presence of an amplification product of the proper size (visualized, for example by gel electrophoresis alongside nucleic acid size markers followed by ethidium bromide staining of the gel, according to standard methods) will indicate the presence of the marker in the sample. In some instances, the PCR product may be further subjected to hybridization analysis or to sequencing to enhance the accuracy of the method. A method of hybridization analysis which can be used is described herein.

#### 9. Use of a Polynucleotide of the Invention as a Marker for Chromosome Mapping:

Because a polynucleotide of the invention is novel, (that is, its sequence is unique), it is useful as a marker for chromosomal mapping. There are a number of methods of chromosomal mapping known in the art. Prominent among them is the variant of the *in situ* hybridization technique known as "Fluorescence *In Situ* Hybridization", or FISH. Details of methods and solutions used for *in situ* hybridization are well-known in the art. There are many variations of the FISH technique itself, however the basic approach is similar in each case. Essentially, *in situ* hybridization of cells, nuclei, or metaphase chromosome spreads is performed with a polynucleotide probe either directly labeled with a fluorochrome, or labeled with a moiety which will be bound by a fluorochrome tagged entity. The hybridized probe is visualized by irradiation of the sample with light in the wavelength which excites fluorescence from the fluorochrome. When combined with standard methods of karyotyping known in the art, this method allows the polynucleotide sequence to be localized to a particular arm of a particular chromosome. Once mapped to a specific chromosome, the location of the novel polynucleotide sequence on that chromosome may be further localized by *in situ* hybridization along with probes specific for known genes or sequences, labeled with other fluorescent tags which allow the differentiation of the signals from the different probes. Such an approach and various adaptations of it allows the localization of the novel gene relative to a known gene. Methods of generating and using fluorescence-labeled polynucleotide probes for FISH and chromosome mapping are known in the art (for example, see Malcolm et al., 1981, Ann. Hum. Genet., 45:134; Bar-Am et al., 1992, Genes. Chromosomes & Cancer, 4:314; Pinkel et al., 1988, Proc. Natl. Acad. Sci. USA, 85:9138; U.S. Patent No. 5,728,527). Additional variations of the chromosome mapping method utilize a PCR approach (Dionne et al., 1990, BioTechniques, 8(2):190 and Iggo et al., 1989, Proc. Natl. Acad. Sci. USA, 86:6211).

In addition to being able to determine the chromosomal location of the novel polynucleotide,

similar technology, in which FISH is combined with flow cytometry, will allow the polynucleotide of the invention to be used to sort chromosomes, nuclei, or whole cells containing various dosages (i.e., gene copy numbers) of the gene encoding that polynucleotide (Hulfdin et al., 1998, Nuc. Acids Res., 26:3651). The novel polypeptide may also be useful as a diagnostic indicator of a disease, including but not limited to those listed in Table I (Kuo et al., 1990, Am. J. Hum. Genet., 47:A119).

#### 10. Use of a Polynucleotide of the Invention as a Marker for Analysis of Forensic Materials

Forensic science depends heavily on methods for determining the source of various compounds associated with criminal activity. In particular, the identification of individuals involved in criminal activity through analysis of substances found at the crime scenes is critical. Such identification is possible with genetic typing, which involves the determination of the genotype of an individual with regard to loci which are polymorphic within the population. As used herein, "polymorphic" refers to a gene or other segment of DNA which shows nucleotide sequence variability from individual to individual. The use of PCR techniques and nucleotide probes to detect even single nucleotide changes in a polynucleotide sequence has revolutionized the field of forensic serology (see Reynolds and Sensabaugh, 1991, Anal. Chem., 63:2). For an example of polymorphisms useful for forensic identification and methods of typing samples with regard to those polymorphisms, see U.S. Patent # 5,273,883.

If a polynucleotide of the invention is found to have nucleotide sequence variation among individuals within a population, it may be useful in the analysis of forensic samples. There are a number of methods known to those skilled in the art for typing nucleic acids with regard to polymorphisms. It should be understood that any such method is acceptable according to the invention. One particular method is termed the "reverse dot blot" method. The basic steps involved are: 1) oligonucleotides bearing the sequences of various polymorphic forms of the polynucleotide region to be analyzed are bound to membranes; 2) labeled, PCR-amplified fragments, derived from the sample to be genotyped, and corresponding to the polymorphic region ("target DNA") are allowed to hybridize to the bound oligonucleotides under conditions which only allow the hybridization of molecules with 100% complementary sequences; 3) unbound target DNA is removed; and 4) hybridized molecules are detected.

The specific genotype of the individual from whom the target sample was obtained (amplified), with regard to the polymorphic region of a polynucleotide of the invention, may thus be determined by screening a panel of probes containing the known polymorphic sequence variations of

that region. It should be understood that the hybridization conditions may be adjusted by one of skill in the art so that limited amounts of non-complementarity, including single base mismatches, may be detected with this method.

## 5 Q. Pharmaceutical Compositions--Prevention and Treatment

### 1. Administration of Pharmaceutical Compositions

Administration of pharmaceutical compositions is accomplished orally or parenterally.

Methods of parenteral delivery include topical, intra-arterial (directly to the tumor), intramuscular, 10 subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal administration. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carrier preparations which can be used pharmaceutically.

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers 15 enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable 20 excipients are carbohydrate or protein fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethyl cellulose; and gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such 25 as sodium alginate.

Dragee cores are provided with suitable coatings such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize 30 the quantity of active compound, ie, dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders such as lactose or

starches, lubricants such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations for parenteral administration include aqueous solutions of active compounds. For injection, the pharmaceutical compositions of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated or used in the formulation. Such penetrants are generally known in the art.

## 2. Manufacture and Storage

The pharmaceutical compositions of the present invention may be manufactured in a manner that known in the art, e.g. by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc... Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder in 1mM-50 mM histidine, 0.1%-2% sucrose, 2%-7% mannitol at a PhRange of 4.5 to 5.5 that is combined with buffer prior to use.

After pharmaceutical compositions comprising a compound of the invention formulated in a acceptable carrier have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition with information including amount, frequency and method of administration.

## 3. Therapeutically Effective Dose

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose.

The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be  
5 use to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of protein or its antibodies, antagonists, or inhibitors which ameliorate the symptoms or conditions. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, eg, ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose  
10 lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animals studies is used in formulating a range of dosage for human use. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with  
15 little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage is chosen by the individual physician in view of the patient to be treated. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Additional factors which may be taken into account include the severity of the  
20 disease state; age, weight and gender of the patient; diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long acting pharmaceutical compositions might be administered every 3 to 4 days, every week, or once every two weeks depending on a half-life and clearance rate of the particular formulation.

Dosage amounts may vary from 0.1 to 100,000 micrograms per person per day, for example,  
25  $\mu$ g, 10 $\mu$ g, 100 $\mu$ g, 500  $\mu$ g, 1mg, 10mg, and even up to a total dose of about 1g per person per day, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature. See U.S. Patent Nos. 4,657,760; 5,206,344; or 5,225,212, hereby incorporated by reference. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotide or polypeptides will be specific  
30 to particular cells, conditions, locations, etc...

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way

whatsoever.

The disclosures of all patents, applications, and publications mentioned above and below, including U.S. Ser. No. 60/342,603, are hereby expressly incorporated by reference.

5

## EXAMPLES

### 1. Establishment of an Association Between a Given Polynucleotide Sequence and Diabetes

10 A polynucleotide sequence according to the invention containing a mutation which is believed to be associated with a disease, can be statistically linked to that disease by linkage analysis. An animal model system exhibiting a particular phenotypic defect that is characteristic of the disease of interest is selected. A series of genetic crosses is performed in this animal model system between individuals having an observable mutant phenotype and normal individuals of a control strain. At least one disease-related locus or a chromosomal marker that does not comprise a disease related locus is  
15 used as a marker in these crosses. If a statistically significant pattern of non-random assortment of the mutant trait with a marker locus is observed, the trait is linked to the marker locus.

Similarly, linkage analysis can be performed on an existing human or other mammalian pedigree. According to this method, numerous genetic loci from affected and unaffected family members are compared. Non-random assortment of a given genetic marker between affected and  
20 unaffected family members relative to the distributions observed for other genetic loci indicates that the marker (for example, a variant isoform of a gene) either contributes to the disease or is in physical proximity to another that does so.

If either approach demonstrates a non-random assortment of the disease-related phenotype with a marker locus, this is indicative of an association between the gene underlying the defect and  
25 that locus. Because the strength of any conclusion drawn from linkage analysis is statistically-based, the accuracy of the results is thought to be proportional to the number of crosses or family members and genetic loci analyzed.

### 2. Screening Assay For a Disease

30 A polynucleotide sequence according to the invention can be used as a marker for a normal phenotype or for a phenotype associated with a disease of interest.

If it can be demonstrated by the methods of phenotyping, described above, that a particular sequence is associated with a disease phenotype, this sequence can be used as a marker for a

particular disease. A sequence of interest can be used as a probe to screen genomic DNA from individuals by Southern blot analysis according to the method described above. If the sequence of interest is detected by Southern blot analysis, and the presence of this sequence is confirmed by direct sequencing, it can be concluded that the individual from which the genomic DNA has been isolated has an increased frequency for the development of the disease for which the sequence is a marker.

The marker can also be used as a disease indicator according to the method of PCR. A genomic DNA sample of interest can be analyzed in a PCR reaction wherein one of the primers contains the marker sequence. If the marker sequence is present in the sample DNA, a PCR product will be produced. Alternatively, the PCR primers can be designed such that they amplify a region containing the marker sequence. The amplified product can be analyzed by hybridization methods, described above, to determine the presence of the sequence of interest.

### 3. Use of a Given Polynucleotide as a Target for Drug Screening

A polynucleotide according to the invention, containing a mutation which is believed to be associated with a disease can be used a target for drug screening.

One method of drug screening utilizes eukaryotic or procaryotic host cells which are stably transformed with a polynucleotide according to the invention and either exhibit a particular phenotype characteristic of the presence of the polynucleotide or express a polypeptide or fragment encoded by the polynucleotide. Such cells, either in viable or fixed form, can be used for standard competitive binding assays. In particular, these cells can be used to measure formation of a complex comprising the protein product or fragment of a polynucleotide according to the invention and the agent being tested. Alternatively, these cells can be used to determine if the formation of a complex between the protein product or fragment of a polynucleotide according to the invention and a known ligand is interfered with by an agent being tested.

An alternative method for drug screening involves using of eukaryotic cell lines or cells (such as described above) which contain a polynucleotide according to the invention that produces a defective protein. According to this method, the host cell lines or cells are grown in the presence of a test drug. The rate of growth of the host cells is measured to determine if the compound is capable of regulating the growth of cells expressing a nonfunctional protein product of the polynucleotide according to the invention. Preferably, a drug that is useful according to the invention will increase or decrease the growth rate of a cell by at least 10%. Alternatively, the ability of the test compound to restore the function of the mutant gene protein by at least 10% can be measured by using an appropriate *in vitro* assay for function of the protein product of a gene (as described in Section F

entitled "Identification and Characterization of Polymorphisms"). If the host cell lines or cells express a protein product of a gene that exhibits an aberrant pattern of cellular localization, the ability of the test compound to alter the cellular localization of the protein by at least 10% will be determined. Changes in the cellular localization of a protein of interest will be detected by performing cellular fractionation studies with biosynthetically labeled cells. Alternatively, the cellular localization of a protein of interest can be determined by immunocytochemical methods well known in the art.

A method of drug screening may also involve the use of host eukaryotic cell lines or cells (described above) which have an altered gene that demonstrates an aberrant pattern of expression. By aberrant pattern of expression is meant the level of expression is either abnormally high or low, or the temporal pattern of expression is different from that of the wild type gene. The ability of a test drug to alter the expression of a mutant form of a gene by at least 10% can be measured by Northern blot analysis, S1 nuclease analysis, primer extension or Rnase protection assays, as described above. Alternatively, if a mutant form of a gene contains a polymorphism in the promoter region of a gene, cells can be engineered to express a reporter construct comprising a mutant gene promoter driving expression of a reporter gene (e.g. CAT, luciferase, green fluorescent protein). These cells can be grown in the presence of a test compound and the ability of a test compound to alter the level of activity of the mutant gene promoter can be determined by standard assays for each reporter gene which are well known in the art.

A transgenic animal whose genomic DNA contains a polynucleotide associated with a particular phenotypic defect that is characteristic of the disease of interest, and a normal, control animal (not containing the polynucleotide) can be treated with a candidate drug according to the invention. The ability of a candidate drug to ameliorate symptoms of the disease, by at least 10%, will be analyzed by assessing the disease symptoms and their amelioration.

#### 4. Selection of Osteoarthritis Candidate Gene Set

Genes involved in osteoarthritis

Key pathogenic processes involved in osteoarthritis are:

1. chondrocyte differentiation, development, apoptosis and signalling
2. cartilage components and synthesis : proteoglycans, hyaluronan synthases, extracellular matrix molecules



3. cartilage degradation: cathepsin proteases and matrix metalloproteinases, their inhibitors
4. bone remodelling signals (e.g. RANK/RANKL): BMPs, TGFbeta, interleukins, their receptors and antagonists, downstream signaling.
5. synovial fluid components
- 5 6. systemic factors influencing bone and cartilage remodelling: leptin, estrogen, progesterone, inflammatory cytokines, retinoic acid

Polymorphisms at the following genes have been reported in the literature to be involved with increased risk of osteoarthritis. They include components of the extracellular matrix, and bone-remodelling signalling components (Table 2)

With the aim of expanding and improving on the current limited knowledge of osteoarthritis genetic predisposition, we have collected over 500 candidate bone and cartilage remodelling genes using the following methods:

15

1. extensive literature search for genes involved in relevant biochemical pathways and physiological processes
2. analysis and comparisons of cDNA libraries within the Incyte Lifeseq® database from relevant normal and diseased tissues and *in vitro* modelling systems
3. co-expression analysis using Incyte's "Guilt by Association" algorithm which identifies novel genes in key biochemical pathways by comparing the expression patterns of genes within the Lifeseq® database

25

#### 5. Polymorphisms in Genes Associated with Osteoarthritis

The osteoarthritis candidate gene list was compiled using gene or gene sequences selected from literature sources, using sequence homology, library subtraction and expression analysis.

Expression analysis was performed using "quilt-by-association" queries to identify Incyte-novel and known genes not previously associated with diabetes which have similar expression patterns to genes known to be involved in diabetes or related conditions. Guilt-by-association analysis was performed as described in Walker et al. 1999 Genome Res 9:1198; Walker et al. 1999 Ismb :282; and US Patent Application 09/226,994 entitled "Insulin-Synthesis Genes" (Atty Docket No: PB-0008

US) filed January 7, 1999, all of which are incorporated by reference.

Polymorphism discovery was by fSSCP as described in section F "Identification and Characterization of Polymorphisms", subsection b5 for polymorphisms referred to in Table 3 for source wetSNPs. Polymorphisms referred to as source isSNPs were discovered as described in section F "Identification and Characterization of Polymorphisms", subsection a. Polymorphisms referred to as source dbSNPs are polymorphisms in public genomic sequence where gene structure is unknown. The polymorphisms were mapped to cDNA sequences in the LifeSeqGold database (Incyte) to identify gene identity.

## **6. Frequency of Polymorphisms in Diabetes Associated Genes and Polynucleotides in Various Populations**

Polymorphisms identified in EXAMPLES 4 and 5 were genotyped against populations described below by fSSCP or FP-TDI as described above. The results of the population frequency studies are given in Table 2.

Two panels of human DNA have been developed to support the identification of frequent SNPs within an ethnically diverse population. The genomic Human Diversity Panel will be used where full genomic structure is available, and allows screening of the open reading frame of the gene, including splice junctions. In instances where genomic structure for selected candidate genes may not be available, a cDNA version of the HDP Screening Panel permits screening of the open reading frame of the gene.

This DNA panel is derived from 47 consented individuals from four ethnic groups (Caucasian, African-American, Asian and Hispanic). The panel is sufficiently sized to enable identification of 95% of SNPs with allele population frequencies  $\geq 5\%$ . Comparable utility of our panel with the NIH Diversity panel was demonstrated by parallel screening of 90 kilobases of coding sequence from each panel.

A cDNA counterpart to our Human Diversity Panel has been generated from lymphoblastoid cell lines to obviate the need for intron/exon structure in 50% of human genes. In the absence of genomic structure, this methodology will be employed to screen the entire open reading frame of the gene.

Various modifications and variations of the described compositions, methods, and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with certain embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific

embodiments. Nor should the description of such embodiments be considered exhaustive or limit the invention to the precise forms disclosed. Furthermore, elements from one embodiment can be readily recombined with elements from one or more other embodiments. Such combinations can form a number of embodiments within the scope of the invention. It is intended that the scope of the  
5 invention be defined by the following claims and their equivalents.

TABLE 1

AACT

Full name : alpha-1-antichymotrypsin

Link : AACT\_link\_cdna

Subsequence	GB:AACT	1	1520	#1		
CDS	GB:AACT.1	1302 bp		#2		
ORF	12	1313				
Allele	GB:AACT	1	36	36	A>G	
	source	isSNP	SNP00027203			
	consequence	GB:AACT.1	2	Missense	9-9	A>T
Allele	GB:AACT	1	269	269	A>G	
	source	isSNP	SNP00073834			
	consequence	GB:AACT.1	2	Silent	86-86	F
Allele	GB:AACT	1	830	830	A>G	
	source	isSNP	SNP00047132			
	consequence	GB:AACT.1	2	Silent	273-273	S
Allele	GB:AACT	1	836	836	A>G	
	source	isSNP	SNP00043844			
	consequence	GB:AACT.1	2	Silent	275-275	L
Allele	GB:AACT	1	837	837	A>G	
	source	isSNP	SNP00101207			
	consequence	GB:AACT.1	2	Missense	276-276	F>L
Allele	GB:AACT	1	848	848	A>G	
	source	isSNP	SNP00101208			
	consequence	GB:AACT.1	2	Silent	279-279	P
Allele	GB:AACT	1	854	854	A>G	
	source	isSNP	SNP00052361			
	consequence	GB:AACT.1	2	Silent	281-281	Q
Allele	GB:AACT	1	947	947	G>T	
	source	isSNP	SNP00059862			
	consequence	GB:AACT.1	2	Stop	312-312	
Allele	GB:AACT	1	1227	1227	A>G	
	source	isSNP	SNP00046872			
	consequence	GB:AACT.1	2	Missense	406-406	T>A

GIF AACT-cdna-fwd.gif

Link : FL\_2114865\_link\_genomic

Subsequence	GB:AL049839_2	1	214520	#3		
Subsequence	AACT_mrna_build.1	59531	69154	#4		
Subsequence	AACT_cds.2	59542	67448	#5		
CDS	AACT_cds.2	651 bp	2 exons	#5		
exon	59542	60184				
exon	67441	67448				
mRNA	AACT_mrna_build.1	1523 bp	4 exons	#4		
exon	59531	60184				
exon	64295	64568				
exon	67441	67591				
exon	68711	69154				
Allele	GB:AL049839_2	3	59566	59566	A>G	
	source	isSNP	SNP00027203			
	source	wetSNP	GB:AL049839_2.v59566.G>A			
	consequence	AACT_cds.2	5	Missense	9-9	A>T
Allele	GB:AL049839_2	3	59799	59799	A>G	
	source	isSNP	SNP00073834			
	consequence	AACT_cds.2	5	Silent	86-86	F
Allele	GB:AL049839_2	3	59844	59844	A>G	

TABLE 1 (Cont.)

	source	isSNP	SNP00005018			
	consequence	AACT_cds.2	5	Silent	101-101	K
Allele	GB:AL049839_2	3	60144	60144 A>G		
	source	isSNP	SNP00093217			
	consequence	AACT_cds.2	5	Silent	201-201	S
Allele	GB:AL049839_2	3	64470	64470 A>G		
	source	isSNP	SNP00047132			
	consequence	AACT_cds.2	5	Intron		
Allele	GB:AL049839_2	3	64476	64476 A>G		
	source	isSNP	SNP00043844			
	consequence	AACT_cds.2	5	Intron		
Allele	GB:AL049839_2	3	64477	64477 A>G		
	source	isSNP	SNP00101207			
	consequence	AACT_cds.2	5	Intron		
Allele	GB:AL049839_2	3	64488	64488 A>G		
	source	isSNP	SNP00101208			
	consequence	AACT_cds.2	5	Intron		
Allele	GB:AL049839_2	3	64494	64494 A>G		
	source	isSNP	SNP00052361			
	consequence	AACT_cds.2	5	Intron		
Allele	GB:AL049839_2	3	65434	65434 A>G		
	source	isSNP	SNP00052361			
	consequence	AACT_cds.2	5	Intron		
Allele	GB:AL049839_2	3	65440	65440 A>G		
	source	isSNP	SNP00101208			
	consequence	AACT_cds.2	5	Intron		
Allele	GB:AL049839_2	3	65451	65451 A>G		
	source	isSNP	SNP00101207			
	consequence	AACT_cds.2	5	Intron		
Allele	GB:AL049839_2	3	65452	65452 A>G		
	source	isSNP	SNP00043844			
	consequence	AACT_cds.2	5	Intron		
Allele	GB:AL049839_2	3	65458	65458 A>G		
	source	isSNP	SNP00047132			
	consequence	AACT_cds.2	5	Intron		
Allele	GB:AL049839_2	3	68858	68858 A>G		
	source	isSNP	SNP00046872			
	consequence	AACT_cds.2	5	3'		
Allele	GB:AL049839_2	3	68882	68882 A>G		
	source	wetSNP	GB:AL049839_2.v68882.A>G			
	consequence	AACT_cds.2	5	3'		

GIF AACT-genomic-fwd.gif

## ABL1

Full name : v-abl Abelson murine leukemia viral oncogene homolog 1

Link : ABL1\_link\_cdna

Subsequence	GB:NM_005157	1	5744	#6		
CDS	GB:NM_005157.1	3393 bp		#7		
ORF	365	3757				
Allele	GB:NM_005157	6	1916	1916	C>G	
	source	isSNP	SNP00046020			
	consequence	GB:NM_005157.1	7	Missense	518-518	A>P
Allele	GB:NM_005157	6	2716	2716	C>G	

TABLE 1 (Cont.)

	source	isSNP	SNP00068702				
	consequence	GB:NM_005157.1	7	Silent	784-784	P	
Allele	GB:NM_005157	6	3625	3625	A>G		
	source	isSNP	SNP00098956				
	consequence	GB:NM_005157.1	7	Silent	1087-1087	I	
Allele	GB:NM_005157	6	3688	3688	A>G		
	source	isSNP	SNP00012765				
	consequence	GB:NM_005157.1	7	Silent	1108-1108	P	
Allele	GB:NM_005157	6	3894	3894	C>G		
	source	isSNP	SNP00046021				
	consequence	GB:NM_005157.1	7	3'			
Allele	GB:NM_005157	6	4612	4612	A>G		
	source	isSNP	SNP00051628				
	consequence	GB:NM_005157.1	7	3'			
Allele	GB:NM_005157	6	5512	5512	A>G		
	source	isSNP	SNP00012768				
	consequence	GB:NM_005157.1	7	3'			
GIF ABL1-cdna-fwd.gif							
Link : ABL1_link_genomic							
Subsequence	ABL1_cds.1	73887	116507		#8		
Subsequence	ABL1_cds.2	29132	116507		#9		
Subsequence	GB:U07561_1	1	35962	#10			
Subsequence	GB:U07563_1	36063	120601		#11		
Subsequence	ABL1_mrna_build.1	73506	118495		#12		
Subsequence	ABL1_mrna_build.2	28792	116507		#13		
Subsequence	ABL1_mrna_build.3	73724	116507		#14		
CDS	ABL1_cds.1	3393 bp	11 exons	#8			
exon	73887	73965					
exon	85951	86124					
exon	86688	86983					
exon	94650	94922					
exon	104016	104100					
exon	104747	104924					
exon	106755	106939					
exon	109237	109389					
exon	110890	110979					
exon	111322	111486					
exon	114793	116507					
CDS	ABL1_cds.2	3450 bp	11 exons	#9			
exon	29132	29267					
exon	85951	86124					
exon	86688	86983					
exon	94650	94922					
exon	104016	104100					
exon	104747	104924					
exon	106755	106939					
exon	109237	109389					
exon	110890	110979					
exon	111322	111486					
exon	114793	116507					
mRNA	ABL1_mrna_build.1	5762 bp	11 exons	#12			
exon	73506	73965					
exon	85951	86124					
exon	86688	86983					
exon	94650	94922					

TABLE 1 (Cont.)

exon	104016	104100			
exon	104747	104924			
exon	106755	106939			
exon	109237	109389			
exon	110890	110979			
exon	111322	111486			
exon	114793	118495			
mRNA	ABL1_mrna_build.2	3787 bp	11 exons	#13	
exon	28792	29267			
exon	85954	86124			
exon	86688	86983			
exon	94650	94922			
exon	104016	104100			
exon	104747	104924			
exon	106755	106939			
exon	109237	109389			
exon	110890	110979			
exon	111322	111486			
exon	114793	116507			
mRNA	ABL1_mrna_build.3	3556 bp	11 exons	#14	
exon	73724	73965			
exon	85951	86124			
exon	86688	86983			
exon	94650	94922			
exon	104016	104100			
exon	104747	104924			
exon	106755	106939			
exon	109237	109389			
exon	110890	110979			
exon	111322	111486			
exon	114793	116507			
Allele	GB:U07561_1	10	29061	29061	A>G
	source	isSNP	SNP00120072		
	consequence	ABL1_cds.1	8		5'
	consequence	ABL1_cds.2	9		5'
Allele	GB:U07561_1	10	30837	30837	A>G
	source	dbSNP	gnl dbSNP ss642659_allele		
	source	dbSNP	gnl dbSNP ss1045108_allele		
	source	dbSNP	gnl dbSNP ss1044696_allele		
	consequence	ABL1_cds.1	8		5'
	consequence	ABL1_cds.2	9		Intron
Allele	GB:U07563_1	11	35864	35864	A>G
	source	isSNP	SNP00048470		
	consequence	ABL1_cds.1	8		5'
	consequence	ABL1_cds.2	9		Intron
Allele	GB:U07563_1	11	58876	58876	C>G
	source	wetSNP	GB:U07563_1.v58876.C>G		
	consequence	ABL1_cds.1	8		Intron
	consequence	ABL1_cds.2	9		Intron
Allele	GB:U07563_1	11	68640	68640	A>G
	source	wetSNP	GB:U07563_1.v68640.T>C		
	consequence	ABL1_cds.1	8		Intron
	consequence	ABL1_cds.2	9		Intron
Allele	GB:U07563_1	11	74901	74901	A>G
	source	wetSNP	GB:U07563_1.v74901.A>G		

TABLE 1 (Cont.)

	consequence	ABL1_cds.1	8	Silent	499-499	E
	consequence	ABL1_cds.2	9	Silent	518-518	E
Allele	GB:U07563_1	11	75298	75298	C>G	
	source	isSNP	SNP00046020			
	consequence	ABL1_cds.1	8	Missense	518-518	A>P
	consequence	ABL1_cds.2	9	Missense	537-537	A>P
Allele	GB:U07563_1	11	78921	78921	A>G	
	source	wetSNP	GB:U07563_1.v78921.G>A			
	consequence	ABL1_cds.1	8	Silent	623-623	E
	consequence	ABL1_cds.2	9	Silent	642-642	E
Allele	GB:U07563_1	11	79239	79239	A>G	
	source	wetSNP	GB:U07563_1.v79239.G>A			
	consequence	ABL1_cds.1	8	Silent	729-729	T
	consequence	ABL1_cds.2	9	Silent	748-748	T
Allele	GB:U07563_1	11	79404	79404	C>G	
	source	isSNP	SNP00068702			
	source	wetSNP	GB:U07563_1.v79404.C>G			
	consequence	ABL1_cds.1	8	Silent	784-784	P
	consequence	ABL1_cds.2	9	Silent	803-803	P
Allele	GB:U07563_1	11	79657	79657	A>G	
	source	wetSNP	GB:U07563_1.v79657.C>T			
	consequence	ABL1_cds.1	8	Missense	869-869	P>S
	consequence	ABL1_cds.2	9	Missense	888-888	P>S
Allele	GB:U07563_1	11	79750	79750	A>G	
	source	wetSNP	GB:U07563_1.v79750.C>T			
	consequence	ABL1_cds.1	8	Missense	900-900	P>S
	consequence	ABL1_cds.2	9	Missense	919-919	P>S
Allele	GB:U07563_1	11	80313	80313	A>G	
	source	isSNP	SNP00098956			
	consequence	ABL1_cds.1	8	Silent	1087-1087	I
	consequence	ABL1_cds.2	9	Silent	1106-1106	I
Allele	GB:U07563_1	11	80376	80376	A>G	
	source	isSNP	SNP00012765			
	source	wetSNP	GB:U07563_1.v80376.G>A			
	consequence	ABL1_cds.1	8	Silent	1108-1108	P
	consequence	ABL1_cds.2	9	Silent	1127-1127	P
Allele	GB:U07563_1	11	80582	80582	C>G	
	source	isSNP	SNP00046021			
	consequence	ABL1_cds.1	8	3'		
	consequence	ABL1_cds.2	9	3'		
Allele	GB:U07563_1	11	81298	81298	A>G	
	source	isSNP	SNP00051628			
	consequence	ABL1_cds.1	8	3'		
	consequence	ABL1_cds.2	9	3'		
Allele	GB:U07563_1	11	81806	81806	A>G	
	source	isSNP	SNP00012766			
	consequence	ABL1_cds.1	8	3'		
	consequence	ABL1_cds.2	9	3'		
Allele	GB:U07563_1	11	82199	82199	A>G	
	source	isSNP	SNP00012768			
	consequence	ABL1_cds.1	8	3'		
	consequence	ABL1_cds.2	9	3'		
GIF ABL1-genomic-fwd.gif						



TABLE 1 (Cont.)

## ADAM9

Full name : a disintegrin and metalloproteinase domain 9

Link : ADAM9\_link\_cdna

Subsequence	GB:HSU41766	1	3865	#15			
CDS	GB:HSU41766.1	2460 bp		#16			
ORF	79	2538					
Allele	GB:HSU41766	15	462	462	G>T		
	source	isSNP	SNP00060630				
	consequence	GB:HSU41766.1	16	Missense	128-128	I>M	
Allele	GB:HSU41766	15	1486	1486	A>G		
	source	isSNP	SNP00122821				
	consequence	GB:HSU41766.1	16	Missense	470-470	G>S	
Allele	GB:HSU41766	15	1580	1580	G>T		
	source	isSNP	SNP00060631				
	consequence	GB:HSU41766.1	16	Missense	501-501	N>T	
Allele	GB:HSU41766	15	2845	2845	A>G		
	source	isSNP	SNP00024957				
	consequence	GB:HSU41766.1	16	3'			
Allele	GB:HSU41766	15	3112	3112	A>G		
	source	isSNP	SNP00122822				
	consequence	GB:HSU41766.1	16	3'			
Allele	GB:HSU41766	15	3703	3703	A>G		
	source	isSNP	SNP00024958				
	consequence	GB:HSU41766.1	16	3'			

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## ADAMTS1

Full name : a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1

Link : ADAMTS1\_link\_cdna

Subsequence	GB:AF060152_1	1	3430	#17			
CDS	GB:AF060152_1.1	2853 bp		#18			
ORF	238	3090					
Allele	GB:AF060152_1	17	140	140	C>G		
	source	isSNP	SNP00109009				
	consequence	GB:AF060152_1.1	18	5'			
Allele	GB:AF060152_1	17	282	282	G>T		
	source	isSNP	SNP00071624				
	consequence	GB:AF060152_1.1	18	Silent	15-15	P	
Allele	GB:AF060152_1	17	768	768	G>T		
	source	isSNP	SNP00069180				
	consequence	GB:AF060152_1.1	18	Silent	177-177	V	
Allele	GB:AF060152_1	17	865	865	C>G		
	source	isSNP	SNP00069181				
	consequence	GB:AF060152_1.1	18	Missense	210-210	P>A	
Allele	GB:AF060152_1	17	1686	1686	A>G		
	source	isSNP	SNP00033973				
	consequence	GB:AF060152_1.1	18	Silent	483-483	P	
Allele	GB:AF060152_1	17	2294	2294	A>G		
	source	isSNP	SNP00109010				
	consequence	GB:AF060152_1.1	18	Missense	686-686	R>H	
Allele	GB:AF060152_1	17	2370	2370	A>G		
	source	isSNP	SNP00033974				

TABLE 1 (Cont.)

	consequence	GB:AF060152_1.1	18	Silent	711-711	S
Allele	GB:AF060152_1	17	2958	2958	A>G	
	source	isSNP	SNP00033975			
	consequence	GB:AF060152_1.1	18	Silent	907-907	C

GIF ADAMTS1-cdna-fwd.gif

## ADAMTS4

Full name : a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 4

Link : ADAMTS4\_link\_cdna

Subsequence	GB:NM_005099_1	1	4301	#19
CDS	GB:NM_005099_1.1	2514 bp		#20
ORF	401	2914		
Allele	GB:NM_005099_1	19	2970	2970 A>G
	source	isSNP	SNP00022951	
	consequence	GB:NM_005099_1.1	20	3'
Allele	GB:NM_005099_1	19	3529	3529 A>G
	source	dbSNP	gnl dbSNP ss610462_allele	
	consequence	GB:NM_005099_1.1	20	3'
Allele	GB:NM_005099_1	19	3533	3533 A>G
	source	dbSNP	gnl dbSNP ss722414_allele	
	source	dbSNP	gnl dbSNP ss999631_allele	
	consequence	GB:NM_005099_1.1	20	3'
Allele	GB:NM_005099_1	19	3855	3855 A>G
	source	dbSNP	gnl dbSNP ss1298908_allele	
	consequence	GB:NM_005099_1.1	20	3'

GIF ADAMTS4-cdna-fwd.gif

## AGC1

Full name : aggrecan 1

Link : AGC1\_link\_cdna

Subsequence	GB:HUMAGPRO	1	7137	#21
CDS	GB:HUMAGPRO.1	6951 bp		#22
ORF	61	7011		
Allele	GB:HUMAGPRO	21	6495	6495 G>T
	source	isSNP	SNP00010327	
	consequence	GB:HUMAGPRO.1	22	Silent
				2145-2145 A

GIF AGC1-cdna-fwd.gif

## ANK

Full name : human homolog of mouse ank gene

Link : ANK\_fl\_link\_cdna

Subsequence	FN:3255641CB1	1	1481	#23
CDS	FN:3255641CB1.1	1338 bp		#24
ORF	106	1443		
Allele	FN:3255641CB1	23	258	258 A>G
	source	isSNP	SNP00073561	
	consequence	FN:3255641CB1.1	24	Silent
				51-51 A
Allele	FN:3255641CB1	23	1048	1048 C>G
		148		

TABLE 1 (Cont.)

	source	isSNP	SNP00036339				
	consequence	FN:3255641CB1.1	24	Missense	315-315	A>P	
Allele	FN:3255641CB1	23	1106	1106	A>G		
	source	isSNP	SNP00075037				
	consequence	FN:3255641CB1.1	24	Missense	334-334	V>A	
Allele	FN:3255641CB1	23	1373	1373	A>G		
	source	isSNP	SNP00045819				
	consequence	FN:3255641CB1.1	24	Missense	423-423	S>F	
GIF ANK-cdna-fwd.gif							
Link : ANK_link_cdna							
Subsequence	GB:AF274753_1	1	1568	#25			
CDS	GB:AF274753_1.1	1479 bp		#26			
ORF	69	1547					
Allele	GB:AF274753_1	25	362	362	A>G		
	source	isSNP	SNP00073561				
	consequence	GB:AF274753_1.1	26	Silent	98-98	A	
Allele	GB:AF274753_1	25	1152	1152	C>G		
	source	isSNP	SNP00036339				
	consequence	GB:AF274753_1.1	26	Missense	362-362	A>P	
Allele	GB:AF274753_1	25	1210	1210	A>G		
	source	isSNP	SNP00075037				
	consequence	GB:AF274753_1.1	26	Missense	381-381	V>A	
Allele	GB:AF274753_1	25	1477	1477	A>G		
	source	isSNP	SNP00045819				
	consequence	GB:AF274753_1.1	26	Missense	470-470	S>F	
GIF ANK-cdna-fwd.gif							
Link : ANK_link_genomic							
Subsequence	ANK_cds.1	26332	84281	#27			
Subsequence	GBI:AC016575_6_000010	1	605	#28			
Subsequence	GB:AC026437_2	706	92528	#29			
Subsequence	ANK_mrna_build.1	308	85658	#30			
Subsequence	ANK_cds.2	272	84281	#31			
CDS	ANK_cds.1	1338 bp	11 exons	#27			
exon	26332	26503					
exon	36882	37000					
exon	39535	39618					
exon	44240	44410					
exon	46173	46307					
exon	49517	49609					
exon	53557	53652					
exon	78643	78772					
exon	81811	81934					
exon	82505	82604					
exon	84168	84281					
CDS	ANK_cds.2	1479 bp	12 exons	#31			
exon	272	367					
exon	26287	26503					
exon	36882	37000					
exon	39535	39618					
exon	44240	44410					
exon	46173	46307					
exon	49517	49609					
exon	53557	53652					
exon	78643	78772					
exon	81811	81934					

TABLE 1 (Cont.)

exon	82505	82604				
exon	84168	84281				
mRNA	ANK_mrna_build.1	2820 bp	12 exons	#30		
exon	308	367				
exon	26287	26503				
exon	36882	37000				
exon	39535	39618				
exon	44240	44410				
exon	46173	46307				
exon	49517	49609				
exon	53557	53652				
exon	78643	78772				
exon	81811	81934				
exon	82505	82604				
exon	84168	85658				
Allele	GB:AC026437_2	29	8413	8413	C>G	
	source	dbSNP gn1 dbSNP ss95678_allele				
	consequence	ANK_cds.1	27	5'		
	consequence	ANK_cds.2	31	Intron		
Allele	GB:AC026437_2	29	14825	14825	A>G	
	source	dbSNP gn1 dbSNP ss619053_allele				
	source	dbSNP gn1 dbSNP ss1002004_allele				
	source	dbSNP gn1 dbSNP ss227983_allele				
	source	dbSNP gn1 dbSNP ss324626_allele				
	consequence	ANK_cds.1	27	5'		
	consequence	ANK_cds.2	31	Intron		
Allele	GB:AC026437_2	29	25779	25779	A>G	
	source	wetSNP	GB:AC026437_2.v25779.C>T			
	consequence	ANK_cds.1	27	Silent	51-51	A
	consequence	ANK_cds.2	31	Silent	98-98	A
Allele	GB:AC026437_2	29	25807	25807	A>G	
	source	isSNP SNP00104502				
	source	wetSNP	GB:AC026437_2.v25807.G>A			
	consequence	ANK_cds.1	27	Intron		
	consequence	ANK_cds.2	31	Intron		
Allele	GB:AC026437_2	29	26433	26433	A>G	
	source	isSNP SNP00018441				
	consequence	ANK_cds.1	27	Intron		
	consequence	ANK_cds.2	31	Intron		
Allele	GB:AC026437_2	29	30696	30696	A>T	
	source	dbSNP gn1 dbSNP ss1016631_allele				
	source	dbSNP gn1 dbSNP ss389763_allele				
	consequence	ANK_cds.1	27	Intron		
	consequence	ANK_cds.2	31	Intron		
Allele	GB:AC026437_2	29	34277	34277	A>G	
	source	isSNP SNP00101566				
	consequence	ANK_cds.1	27	Intron		
	consequence	ANK_cds.2	31	Intron		
Allele	GB:AC026437_2	29	36172	36172	A>G	
	source	wetSNP	GB:AC026437_2.v36172.T>C			
	consequence	ANK_cds.1	27	Intron		
	consequence	ANK_cds.2	31	Intron		
Allele	GB:AC026437_2	29	37028	37028	G>T	
	source	isSNP SNP00056800				
	consequence	ANK_cds.1	27	Intron		
			150			

TABLE 1 (Cont.)

Allele	consequence	ANK_cds.2	31	Intron		
	GB:AC026437_2	29	37186	37186	G>T	
	source	isSNP	SNP00022144			
Allele	consequence	ANK_cds.1	27	Intron		
	consequence	ANK_cds.2	31	Intron		
	GB:AC026437_2	29	37205	37205	A>G	
Allele	source	isSNP	SNP00022143			
	consequence	ANK_cds.1	27	Intron		
	consequence	ANK_cds.2	31	Intron		
Allele	GB:AC026437_2	29	37340	37340	A>T	
	source	dbSNP	gnl dbSNP ss469809_allele			
	consequence	ANK_cds.1	27	Intron		
Allele	consequence	ANK_cds.2	31	Intron		
	GB:AC026437_2	29	52817	52817	G>T	
	source	wetSNP	GB:AC026437_2.v52817.C>A			
Allele	consequence	ANK_cds.1	27	Intron		
	consequence	ANK_cds.2	31	Intron		
	GB:AC026437_2	29	52899	52899	A>G	
Allele	source	wetSNP	GB:AC026437_2.v52899.A>G			
	consequence	ANK_cds.1	27	Silent	274-274	A
	consequence	ANK_cds.2	31	Silent	321-321	A
Allele	GB:AC026437_2	29	52962	52962	G>T	
	source	wetSNP	GB:AC026437_2.v52962.T>G			
	consequence	ANK_cds.1	27	Intron		
Allele	consequence	ANK_cds.2	31	Intron		
	GB:AC026437_2	29	63950	63950	A>G	
	source	isSNP	SNP00093702			
Allele	consequence	ANK_cds.1	27	Intron		
	consequence	ANK_cds.2	31	Intron		
	GB:AC026437_2	29	78010	78010	C>G	
Allele	source	isSNP	SNP00036339			
	consequence	ANK_cds.1	27	Missense	315-315	A>P
	consequence	ANK_cds.2	31	Missense	362-362	A>P
Allele	GB:AC026437_2	29	78875	78875	A>G	
	source	isSNP	SNP00095793			
	consequence	ANK_cds.1	27	Intron		
Allele	consequence	ANK_cds.2	31	Intron		
	GB:AC026437_2	29	81235	81235	A>G	
	source	wetSNP	GB:AC026437_2.v81235.T>C			
Allele	consequence	ANK_cds.1	27	Intron		
	consequence	ANK_cds.2	31	Intron		
	GB:AC026437_2	29	82852	82852	A>G	
Allele	source	isSNP	SNP00120424			
	consequence	ANK_cds.1	27	Intron		
	consequence	ANK_cds.2	31	Intron		
Allele	GB:AC026437_2	29	83057	83057	A>G	
	source	isSNP	SNP00120425			
	consequence	ANK_cds.1	27	Intron		
Allele	consequence	ANK_cds.2	31	Intron		
	GB:AC026437_2	29	83506	83506	A>G	
	source	isSNP	SNP00045819			
Allele	consequence	ANK_cds.1	27	Missense	423-423	S>F
	consequence	ANK_cds.2	31	Missense	470-470	S>F
	GB:AC026437_2	29	83587	83587	A>G	
Allele	source	wetSNP	GB:AC026437_2.v83587.G>A			
			151			

TABLE 1 (Cont.)

	consequence	ANK_cds.1	27	3'	
	consequence	ANK_cds.2	31	3'	
Allele	GB:AC026437_2	29	83607	83607	A>G
	source	isSNP	SNP00008779		
	source	wetSNP	GB:AC026437_2.v83607.A>G		
	consequence	ANK_cds.1	27	3'	
	consequence	ANK_cds.2	31	3'	
Allele	GB:AC026437_2	29	84086	84086	A>G
	source	isSNP	SNP00012596		
	consequence	ANK_cds.1	27	3'	
	consequence	ANK_cds.2	31	3'	
Allele	GB:AC026437_2	29	84156	84156	A>G
	source	isSNP	SNP00045820		
	consequence	ANK_cds.1	27	3'	
	consequence	ANK_cds.2	31	3'	
Allele	GB:AC026437_2	29	84896	84896	G>T
	source	isSNP	SNP00045822		
	consequence	ANK_cds.1	27	3'	
	consequence	ANK_cds.2	31	3'	

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## BGLAP

Full name : Bone Gla Protein

Link : FL\_104137\_link\_genomic

Subsequence	GB:AC007227_2_104137CD1	35521	34594	#32	
Subsequence	GB:AC007227_2	1	167932	#33	
Subsequence	BGLAP_mrna_build.1	35539	34461	#34	
mRNA	BGLAP_mrna_build.1	451 bp	4 exons	#34	
exon	35539	35458			
exon	35200	35162			
exon	34991	34922			
exon	34720	34461			
CDS	GB:AC007227_2_104137CD1	300 bp	4 exons	#32	
exon	35521	35458			
exon	35200	35162			
exon	34991	34922			
exon	34720	34594			
Allele	GB:AC007227_2	33	34618	34618	C>G
	source	wetSNP	GB:AC007227_2.v34618.G>C		
	consequence	GB:AC007227_2_104137CD1	32	Silent	92-92 A
Allele	GB:AC007227_2	33	34977	34977	G>T
	source	wetSNP	GB:AC007227_2.v34977.G>T		
	consequence	GB:AC007227_2_104137CD1	32	Missense	40-40 Q>K
Allele	GB:AC007227_2	33	35228	35228	C>G
	source	isSNP	SNP00038471		
	consequence	GB:AC007227_2_104137CD1	32	Intron	

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## BGN

Full name : BGN

Link : BGN\_link\_cdna

TABLE 1 (Cont.)

Subsequence	GB:HUMHPGI	1	1685	#35		
CDS	GB:HUMHPGI.1	1107 bp		#36		
ORF	121	1227				
Allele	GB:HUMHPGI	35	70	70	G>T	
	source	isSNP	SNP00011488			
	consequence	GB:HUMHPGI.1	36	5'		
Allele	GB:HUMHPGI	35	261	261	A>G	
	source	isSNP	SNP00011489			
	consequence	GB:HUMHPGI.1	36	Silent	47-47	S
Allele	GB:HUMHPGI	35	660	660	A>G	
	source	isSNP	SNP00011490			
	consequence	GB:HUMHPGI.1	36	Silent	180-180	S
Allele	GB:HUMHPGI	35	1355	1355	A>G	
	source	isSNP	SNP00092805			
	consequence	GB:HUMHPGI.1	36	3'		
GIF BGN-cdna-fwd.gif						
Link : BGN_link_genomic						
Subsequence	GB:U82695	1	76146	#37		
Subsequence	GB:U82695_2540367CD1		18042	21854	#38	
Subsequence	BGN_mrna_build.1	8415	22311	#39		
CDS	GB:U82695_2540367CD1	1107 bp	7 exons	#38		
exon	18042	18279				
exon	18648	18760				
exon	19272	19485				
exon	19938	20048				
exon	20239	20332				
exon	20456	20594				
exon	21657	21854				
mRNA	BGN_mrna_build.1	1684 bp	8 exons	#39		
exon	8415	8523				
exon	18031	18279				
exon	18648	18760				
exon	19272	19485				
exon	19938	20048				
exon	20239	20332				
exon	20456	20594				
exon	21657	22311				
Allele	GB:U82695	37	8484	8484	G>T	
	source	isSNP	SNP00011488			
	consequence	GB:U82695_2540367CD1	38	5'		
Allele	GB:U82695	37	18161	18161	A>G	
	source	wetSNP	GB:U82695.v18161.A>G			
	consequence	GB:U82695_2540367CD1	38	Silent	40-40	E
Allele	GB:U82695	37	18182	18182	A>G	
	source	isSNP	SNP00011489			
	source	wetSNP	GB:U82695.v18182.G>A			
	consequence	GB:U82695_2540367CD1	38	Silent	47-47	S
Allele	GB:U82695	37	18330	18330	A>G	
	source	wetSNP	GB:U82695.v18330.G>A			
	consequence	GB:U82695_2540367CD1	38	Intron		
Allele	GB:U82695	37	18354	18354	A>G	
	source	wetSNP	GB:U82695.v18354.G>A			
	consequence	GB:U82695_2540367CD1	38	Intron		
Allele	GB:U82695	37	19460	19460	A>G	
	source	isSNP	SNP00011490			

TABLE 1 (Cont.)

	source	wetSNP	GB:U82695.v19460.T>C			
	consequence	GB:U82695_2540367CD1	38	Silent		180-180
S						
Allele	GB:U82695	37	21566	21566	G>T	
	source	wetSNP	GB:U82695.v21566.G>T			
	consequence	GB:U82695_2540367CD1	38	Intron		
Allele	GB:U82695	37	21639	21639	A>G	
	source	wetSNP	GB:U82695.v21639.C>T			
	consequence	GB:U82695_2540367CD1	38	Intron		
Allele	GB:U82695	37	21982	21982	A>G	
	source	isSNP	SNP00092805			
	consequence	GB:U82695_2540367CD1	38	3'		
Allele	GB:U82695	37	22172	22172	G>T	
	source	isSNP	SNP00011491			
	consequence	GB:U82695_2540367CD1	38	3'		

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## BHLHB2

Full name : basic helix-loop-helix domain containing, class B, 2

Link : BHLHB2\_link\_cdna

Subsequence	GB:AB004066_1	1	2922	#40		
CDS	GB:AB004066_1.1	1239 bp		#41		
ORF	197	1435				
Allele	GB:AB004066_1	40	196	196	A>G	
	source	isSNP	SNP00062724			
	consequence	GB:AB004066_1.1	41	5'		
Allele	GB:AB004066_1	40	829	829	A>G	
	source	isSNP	SNP00046376			
	consequence	GB:AB004066_1.1	41	Silent		211-211 G
Allele	GB:AB004066_1	40	2070	2070	A>G	
	source	isSNP	SNP00013041			
	consequence	GB:AB004066_1.1	41	3'		
Allele	GB:AB004066_1	40	2323	2323	A>G	
	source	isSNP	SNP00013042			
	consequence	GB:AB004066_1.1	41	3'		

GIF BHLHB2-cdna-fwd.gif

## BMP2

Full name : BMP2

Link : BMP2\_link\_cdna

Subsequence	GB:HUMBMP2A	1	1547	#42		
CDS	GB:HUMBMP2A.1	1191 bp		#43		
ORF	324	1514				
Allele	GB:HUMBMP2A	42	584	584	A>G	
	source	isSNP	SNP00015730			
	consequence	GB:HUMBMP2A.1	43	Silent		87-87 S
Allele	GB:HUMBMP2A	42	760	760	A>G	
	source	isSNP	SNP00015731			
	consequence	GB:HUMBMP2A.1	43	Missense		146-146 T>I
Allele	GB:HUMBMP2A	42	984	984	G>T	
	source	isSNP	SNP00015732			



TABLE 1 (Cont.)

	consequence	GB:HUMBMP2A.1	43	Missense	221-221	H>N
Allele	GB:HUMBMP2A	42 1484 1484	A>G			
	source	isSNP SNP00015733				
	consequence	GB:HUMBMP2A.1	43	Silent	387-387	D
GIF BMP2-cdna-fwd.gif						
Link : FL_3220019_link_genomic						
Subsequence	GB:HS859D4	1 178870	#44			
Subsequence	GB:HS859D4_3220019CD1	176685	167723	#45		
Subsequence	BMP2_mrna_build.1	178252	167687	#46		
mRNA	BMP2_mrna_build.1	1547 bp	3 exons	#46		
exon	178252	177937				
exon	176692	176340				
exon	168564	167687				
CDS	GB:HS859D4_3220019CD1	1188 bp	2 exons	#45		
exon	176685	176340				
exon	168564	167723				
Allele	GB:HS859D4	44 167750	167750	A>G		
	source	isSNP SNP00015733				
	consequence	GB:HS859D4_3220019CD1	45	Silent	387-387	
D						
Allele	GB:HS859D4	44 168250	168250	G>T		
	source	isSNP SNP00015732				
	consequence	GB:HS859D4_3220019CD1	45	Missense	221-221	
H>N						
Allele	GB:HS859D4	44 168341	168341	A>T		
	source	wetSNP GB:HS859D4.v168341.T>A				
	consequence	GB:HS859D4_3220019CD1	45	Missense	190-190	
R>S						
Allele	GB:HS859D4	44 168474	168474	A>G		
	source	isSNP SNP00015731				
	consequence	GB:HS859D4_3220019CD1	45	Missense	146-146	
T>I						
Allele	GB:HS859D4	44 176425	176425	A>G		
	source	isSNP SNP00015730				
	source	wetSNP GB:HS859D4.v176425.T>C				
	consequence	GB:HS859D4_3220019CD1	45	Silent	87-87	S
GIF BMP2-genomic-rev.gif						

## BMP4

Full name : BMP4

Link : BMP4\_link\_cdna

Subsequence	GB:HUMBMP2B	1 1751	#47			
CDS	GB:HUMBMP2B.1	1227 bp	#48			
ORF	395	1621				
Allele	GB:HUMBMP2B	47 308 308	A>G			
	source	isSNP SNP00074676				
	consequence	GB:HUMBMP2B.1	48	5'		
Allele	GB:HUMBMP2B	47 849 849	A>G			
	source	isSNP SNP00000573				
	consequence	GB:HUMBMP2B.1	48	Missense	152-152	V>A

GIF BMP4-cdna-fwd.gif

Link : BMP4\_link\_genomic

Subsequence	GB:HSU43842	1 11233	#49			
		155				

TABLE 1 (Cont.)

Subsequence	GB:HSU43842_1613615CD1	7798	9984	#50
Subsequence	BMP4_mrna_build.1	3207	10117	#51
mRNA	BMP4_mrna_build.1	1751 bp	4 exons	#51
exon	3207	3468		
exon	6620	6744		
exon	7791	8167		
exon	9131	10117		
CDS	GB:HSU43842_1613615CD1	1224 bp	2 exons	#50
exon	7798	8167		
exon	9131	9984		
Allele	GB:HSU43842	49	6665	6665 A>G
source	isSNP	SNP00074676		
consequence	GB:HSU43842_1613615CD1	50	5'	
Allele	GB:HSU43842	49	7752	7752 A>G
source	isSNP	SNP00117542		
consequence	GB:HSU43842_1613615CD1	50	5'	
Allele	GB:HSU43842	49	9215	9215 A>G
source	isSNP	SNP00000573		
source	wetSNP	GB:HSU43842.v9215.C>T		
consequence	GB:HSU43842_1613615CD1	50	Missense	152-152
	A>V			
GIF	BMP4-genomic-fwd.gif			

## BMP6

Full name : BMP6

Link : BMP6\_link\_cdna

Subsequence	GB:HUMTGFBC	1	2923	#52
CDS	GB:HUMTGFBC.1	1542 bp		#53
ORF	160	1701		
Allele	GB:HUMTGFBC	52	1263	1263 C>G
source	isSNP	SNP00069306		
consequence	GB:HUMTGFBC.1	53	Silent	368-368 V
Allele	GB:HUMTGFBC	52	2280	2280 G>T
source	isSNP	SNP00021640		
consequence	GB:HUMTGFBC.1	53	3'	
Allele	GB:HUMTGFBC	52	2436	2436 A>G
source	isSNP	SNP00003240		
consequence	GB:HUMTGFBC.1	53	3'	
Allele	GB:HUMTGFBC	52	2574	2574 A>G
source	isSNP	SNP00021639		
consequence	GB:HUMTGFBC.1	53	3'	
GIF	BMP6-cdna-fwd.gif			

## CAPN4

Full name : calpain, small polypeptide

Link : FL\_508926\_link\_genomic

Subsequence	GB:CH19F24590	1	41369	#54
Subsequence	GB:CH19F24590_3639962CD1		31006	39830 #55
Subsequence	FL_3639962_mrna_build.1	30073	40241	#56
Subsequence	CAPN4_cds.1	31006	39833	#57
mRNA	FL_3639962_mrna_build.1	1309 bp	11 exons	#56
		156		

TABLE 1 (Cont.)

exon	30073	30151							
exon	30991	31214							
exon	32294	32327							
exon	32646	32735							
exon	32903	32960							
exon	33058	33122							
exon	35800	35868							
exon	35970	36048							
exon	36190	36306							
exon	39572	39630							
exon	39807	40241							
CDS	CAPN4_cds.1	717 bp	9 exons			#57			
exon	31006	31214							
exon	32294	32327							
exon	32903	32960							
exon	33058	33122							
exon	35800	35868							
exon	35970	36048							
exon	36190	36306							
exon	39572	39630							
exon	39807	39833							
CDS	GB:CH19F24590_3639962CD1		804 bp		10 exons	#55			
exon	31006	31214							
exon	32294	32327							
exon	32646	32735							
exon	32903	32960							
exon	33058	33122							
exon	35800	35868							
exon	35970	36048							
exon	36190	36306							
exon	39572	39630							
exon	39807	39830							
GIF	CAPN4-genomic-fwd.gif								
CBFA1									
Full name : CBFA1									
Link : CBFA1_link_cdna									
Subsequence	GB:HUMCBFA	1	1411	#58					
CDS	GB:HUMCBFA.2	1323 bp		#59					
ORF	1	1323							
Allele	GB:HUMCBFA	58	260	260	A>G				
	source	isSNP	SNP00063798						
	consequence	GB:HUMCBFA.2	59	Missense	87-87	G>E			
GIF CBFA1-cdna-fwd.gif									
Link : CBFA1_link_genomic									
Subsequence	GB:HSCBFA1S1	1	93	#60					
Subsequence	GB:HSCBFA1S2	194	669	#61					
Subsequence	GB:HSCBFA1S3	770	1034	#62					
Subsequence	GB:HSCBFA1S4	1135	1381	#63					
Subsequence	GB:HSCBFA1S5	1482	1759	#64					
Subsequence	GB:HSCBFA1S6	1860	2081	#65					
Subsequence	GB:HSCBFA1S7	2182	2301	#66					
Subsequence	GB:HSCBFA1S8	2402	3033	#67					
		157							

TABLE 1 (Cont.)

Subsequence	CBFA1_cds.1	28	2948	#68	
CDS	CBFA1_cds.1	1566 bp	8 exons	#68	
exon	28	85			
exon	261	625			
exon	821	977			
exon	1198	1302			
exon	1533	1706			
exon	1881	2042			
exon	2201	2266			
exon	2470	2948			
Allele	GB:HSCBFA1S3	62	177	177	A>G
	source	wetSNP	GB:HSCBFA1S3.v177	C>T	
	consequence	CBFA1_cds.1	68	Silent	183-183 N
Allele	GB:HSCBFA1S8	67	490	490	A>G
	source	wetSNP	GB:HSCBFA1S8.v490	C>T	
	consequence	CBFA1_cds.1	68	Silent	503-503 S
GIF	CBFA1-genomic-fwd.gif				

## CD36

Full name : CD36 Glycoprotein

Link : CD36\_link\_cdna

Subsequence	EM:HSCD3621	1	2216	#69	
Allele	EM:HSCD3621	69	123	123	G>T
	source	isSNP	SNP00011023		
Allele	EM:HSCD3621	69	196	196	A>G
	source	isSNP	SNP00096573		
Allele	EM:HSCD3621	69	230	230	C>G
	source	isSNP	SNP00110263		
Allele	EM:HSCD3621	69	827	827	A>G
	source	isSNP	SNP00115780		
Allele	EM:HSCD3621	69	1332	1332	A>G
	source	isSNP	SNP00096574		

Link : CD36\_link\_genomic

Subsequence	CD36_link_cds.1	2094	6548	#70	
Subsequence	EM:HSCD36G1	101	236	#71	
Subsequence	EM:HSCD36A	338	2898	#72	
Subsequence	EM:HSCD36G4	3000	3220	#73	
Subsequence	EM:HSCD36G5	3322	3529	#74	
Subsequence	EM:HSCD36AA	3631	3999	#75	
Subsequence	EM:HSCD36G7	4101	4252	#76	
Subsequence	EM:HSCD36G8	4354	4460	#77	
Subsequence	EM:HSCD36G9	4562	4691	#78	
Subsequence	EM:HSCD36G10		4793	5042	#79
Subsequence	EM:B74110	5144	5803	#80	
Subsequence	EM:HSCD36G12		5905	6038	#81
Subsequence	EM:HSCD36G13		6140	6252	#82
Subsequence	EM:HSCD36G14		6354	6847	#83
Subsequence	EM:HSCD36G15		6949	7632	#84
Subsequence	CD36_mrna_build.1	136		7602	#85
mRNA	CD36_mrna_build.1	2217 bp	16 exons	#85	
exon	136	206			
exon	1446	1539			
exon	2005	2213			

TABLE 1 (Cont.)

exon	3030	3190							
exon	3352	3499							
exon	3719	3898							
exon	4131	4222							
exon	4384	4430							
exon	4592	4661							
exon	4824	5011							
exon	5265	5383							
exon	5935	6008							
exon	6168	6222							
exon	6384	6548							
exon	6979	7071							
exon	7152	7602							
CDS	CD36_link_cds.1	1419 bp	12 exons	#70					
exon	2094	2213							
exon	3030	3190							
exon	3352	3499							
exon	3719	3898							
exon	4131	4222							
exon	4384	4430							
exon	4592	4661							
exon	4824	5011							
exon	5265	5383							
exon	5935	6008							
exon	6168	6222							
exon	6384	6548							
Allele	EM:HSCD36A	72	1160	1160	G>T				
	source	isSNP	SNP00011023						
	consequence	CD36_link_cds.1	70	5'					
Allele	EM:HSCD36A	72	1698	1698	A>G				
	source	isSNP	SNP00096573						
	consequence	CD36_link_cds.1	70	5'					
Allele	EM:HSCD36A	72	1732	1732	C>G				
	source	isSNP	SNP00110263						
	consequence	CD36_link_cds.1	70	5'					
Allele	EM:HSCD36G4	73	102	102	C>G				
	source	wetSNP	EM:HSCD36G4.v102.G>C						
	consequence	CD36_link_cds.1	70	Missense	64-64	Q>H			
Allele	EM:HSCD36AA	75	232	232	A>G				
	source	isSNP	SNP00115780						
	consequence	CD36_link_cds.1	70	Silent	191-191	P			
Allele	EM:HSCD36G10	79	92	92	A>G				
	source	wetSNP	EM:HSCD36G10.v92.T>C						
	consequence	CD36_link_cds.1	70	Silent	293-293	F			
Allele	EM:B74110	80	193	193	A>G				
	source	isSNP	SNP00096574						
	consequence	CD36_link_cds.1	70	Silent	360-360	L			
Allele	EM:HSCD36G14	83	198	203	AAGTAT>AT				
	source	wetSNP	EM:HSCD36G14.v198.AAGTAT>AT						
	consequence	CD36_link_cds.1	70	3'					
Allele	EM:HSCD36G14	83	421	421	A>G				
	source	isSNP	SNP00041723						
	consequence	CD36_link_cds.1	70	3'					
GIF	CD36-genomic-fwd.gif								

TABLE 1 (Cont.)

CD68

Full name : CD68 antigen

Link : FL\_3777141\_link\_cdna

Subsequence	FN:3777141CB1	1	1558	#86	
CDS	FN:3777141CB1.1	1065 bp		#87	
ORF	75	1139			
Allele	FN:3777141CB1	86	834	834	G>T
	source	isSNP	SNP00006442		
	consequence	FN:3777141CB1.1	87	Missense	254-254 Q>K
Allele	FN:3777141CB1	86	1394	1394	G>T
	source	dbSNP	gnl dbSNP ss450666_allele		
	consequence	FN:3777141CB1.1	87	3'	
Allele	FN:3777141CB1	86	1475	1475	G>T
	source	isSNP	SNP00108664		
	consequence	FN:3777141CB1.1	87	3'	

GIF CD68-cdna-fwd.gif

Link : FL\_1803929\_link\_genomic

Subsequence	GB:AC007421_12	1	95240	#88	
Subsequence	GB:AC007421_12_3777141CD1		92493	90660	#89
Subsequence	FL_3777141_mrna_build.1	92567	90242	#90	
mRNA	FL_3777141_mrna_build.1	1557 bp	6 exons	#90	
exon	92567	92445			
exon	92361	91844			
exon	91705	91586			
exon	91460	91388			
exon	91275	91105			
exon	90793	90242			
CDS	GB:AC007421_12_3777141CD1	1065 bp	6 exons	#89	
exon	92493	92445			
exon	92361	91844			
exon	91705	91586			
exon	91460	91388			
exon	91275	91105			
exon	90793	90660			
Allele	GB:AC007421_12	88	90404	90404	G>T
	source	dbSNP	gnl dbSNP ss450666_allele		
	consequence	GB:AC007421_12_3777141CD1	89	3'	
Allele	GB:AC007421_12	88	90707	90707	A>G
	source	wetSNP	GB:AC007421_12.v90707.C>T		
	consequence	GB:AC007421_12_3777141CD1	89	Missense	
340-340	A>T				
Allele	GB:AC007421_12	88	91388	91388	G>T
	source	wetSNP	GB:AC007421_12.v91388.G>T		
	consequence	GB:AC007421_12_3777141CD1	89	Missense	
254-254	Q>K				
Allele	GB:AC007421_12	88	92357	92357	A>G
	source	wetSNP	GB:AC007421_12.v92357.C>T		
	consequence	GB:AC007421_12_3777141CD1	89	Silent	

18-18 Q

GIF CD68-genomic-rev.gif

TABLE 1 (Cont.)

Full name : cysteine dioxygenase type I

Link : CDO1\_link\_cdna

Subsequence	GB:HHSCYSDIO	1	1556	#91		
CDS	GB:HHSCYSDIO.1	603 bp		#92		
ORF	255	857				
Allele	GB:HHSCYSDIO	91	100	100	A>G	
	source	isSNP	SNP00009024			
	consequence	GB:HHSCYSDIO.1	92	5'		
Allele	GB:HHSCYSDIO	91	737	737	A>G	
	source	isSNP	SNP00048574			
	consequence	GB:HHSCYSDIO.1	92	Silent		161-161 F
Allele	GB:HHSCYSDIO	91	784	784	A>G	
	source	isSNP	SNP00036859			
	consequence	GB:HHSCYSDIO.1	92	Missense		177-177 V>A
Allele	GB:HHSCYSDIO	91	1082	1082	A>G	
	source	isSNP	SNP00107326			
	consequence	GB:HHSCYSDIO.1	92	3'		
Allele	GB:HHSCYSDIO	91	1525	1525	A>G	
	source	isSNP	SNP00036860			
	consequence	GB:HHSCYSDIO.1	92	3'		

GIF CDO1-cdna-fwd.gif

Link : CDO1\_link\_genomic

Subsequence	CDO1_cds.1	1653	4275	#93		
Subsequence	GB:D85778_1	1	2601	#94		
Subsequence	GB:D85779_1	2702	2938	#95		
Subsequence	GB:D85780_1	3039	3525	#96		
Subsequence	GB:D85781_1	3626	4090	#97		
Subsequence	GB:D85782_1	4191	4921	#98		
Subsequence	CDO1_mrna_build.1	1402	4921	#99		
mRNA	CDO1_mrna_build.1	1500 bp		5 exons		#99
exon	1402	1822				
exon	2789	2866				
exon	3178	3332				
exon	3777	3946				
exon	4246	4921				
CDS	CDO1_cds.1	603 bp		5 exons		#93
exon	1653	1822				
exon	2789	2866				
exon	3178	3332				
exon	3777	3946				
exon	4246	4275				
Allele	GB:D85778_1	94	1498	1498	A>G	
	source	isSNP	SNP00009024			
	consequence	CDO1_cds.1	93	5'		
Allele	GB:D85781_1	97	278	278	A>G	
	source	isSNP	SNP00036859			
	consequence	CDO1_cds.1	93	Missense		177-177 V>A
Allele	GB:D85782_1	98	310	310	A>G	
	source	isSNP	SNP00107326			
	consequence	CDO1_cds.1	93	3'		

GIF CDO1-genomic-fwd.gif

TABLE 1 (Cont.)

Link : CGI-52\_link\_cdna

Subsequence	GB:AF151810	1	1414	#100		
CDS	GB:AF151810.1	1080 bp		#101		
ORF	277	1356				
Allele	GB:AF151810	100	1335	1335	A>G	
	source	isSNP	SNP00054191			
	consequence	GB:AF151810.1	101	Silent	353-353	D

GIF CGI-52-cdna-fwd.gif

Link : CGI-52\_link\_genomic

Subsequence	GB:AC023176_7	1	193672	#102		
Subsequence	CGI-52_mrna_build.1	131456	93050	#103		
mRNA	CGI-52_mrna_build.1	1420 bp	7 exons	#103		
exon	131456	131084				
exon	119505	119186				
exon	97592	97445				
exon	96844	96741				
exon	96095	95978				
exon	93964	93912				
exon	93353	93050				
Allele	GB:AC023176_7	102	93129	93129	A>G	
	source	isSNP	SNP00054191			
Allele	GB:AC023176_7	102	93416	93416	A>G	
	source	isSNP	SNP00057212			
Allele	GB:AC023176_7	102	131305	131305	C>G	
	source	isSNP	SNP00069496			

GIF CGI-52-genomic-rev.gif

CHI3L1

Full name : chitinase 3-like 1

Link : CHI3L1\_link\_cdna

Subsequence	GB:NM_001276_1	1	1925	#104		
CDS	GB:NM_001276_1.1	1152 bp		#105		
ORF	127	1278				
Allele	GB:NM_001276_1	104	559	559	A>G	
	source	isSNP	SNP00008252			
	consequence	GB:NM_001276_1.1	105	Missense	145-145	R>G
Allele	GB:NM_001276_1	104	590	590	A>G	
	source	isSNP	SNP00071935			
	consequence	GB:NM_001276_1.1	105	Missense	155-155	K>R
Allele	GB:NM_001276_1	104	646	646	G>T	
	source	isSNP	SNP00022932			
	consequence	GB:NM_001276_1.1	105	Missense	174-174	L>I
Allele	GB:NM_001276_1	104	1300	1300	A>G	
	source	isSNP	SNP00052666			
	consequence	GB:NM_001276_1.1	105	3'		
Allele	GB:NM_001276_1	104	1342	1342	A>G	
	source	isSNP	SNP00072805			
	consequence	GB:NM_001276_1.1	105	3'		
Allele	GB:NM_001276_1	104	1739	1739	A>G	
	source	isSNP	SNP00076686			
	consequence	GB:NM_001276_1.1	105	3'		

GIF CHI3L1-cdna-fwd.gif

Link : CHI3L1\_link\_genomic



TABLE 1 (Cont.)

Subsequence	CHI3L1_cds.1	1295	7276	#106	
Subsequence	CHI3L1_cds.2	1295	7433	#107	
Subsequence	CHI3L1_cds.3	1295	7276	#108	
Subsequence	CHI3L1_cds.4	1295	2802	#109	
Subsequence	GB:Y08374_1	1	1635	#110	
Subsequence	GB:Y08375_1	1736	3186	#111	
Subsequence	GB:Y08376_1	3287	4116	#112	
Subsequence	GB:Y08377_1	4217	5035	#113	
Subsequence	GB:Y08378_1	5136	7923	#114	
Subsequence	CHI3L1_mrna_build.1		1169	7923	#115
Subsequence	CHI3L1_mrna_build.2		1169	7604	#116
mRNA	CHI3L1_mrna_build.2	1355 bp		11 exons	#116
exon	1169	1319			
exon	1572	1601			
exon	2036	2237			
exon	2789	2845			
exon	3606	3756			
exon	4517	4638			
exon	5436	5559			
exon	6069	6251			
exon	6844	6960			
exon	7296	7456			
exon	7548	7604			
CDS	CHI3L1_cds.1	1152 bp	10 exons	#106	
exon	1295	1319			
exon	1572	1601			
exon	2036	2237			
exon	2789	2845			
exon	3606	3756			
exon	4517	4638			
exon	5436	5559			
exon	6069	6251			
exon	6844	6960			
exon	7136	7276			
CDS	CHI3L1_cds.2	1149 bp	10 exons	#107	
exon	1295	1319			
exon	1572	1601			
exon	2036	2237			
exon	2789	2845			
exon	3606	3756			
exon	4517	4638			
exon	5436	5559			
exon	6069	6251			
exon	6844	6960			
exon	7296	7433			
CDS	CHI3L1_cds.3	969 bp	9 exons	#108	
exon	1295	1319			
exon	1572	1601			
exon	2036	2237			
exon	2789	2845			
exon	3606	3756			
exon	4517	4638			
exon	5436	5559			
exon	6844	6960			
exon	7136	7276			

TABLE 1 (Cont.)

mRNA	CHI3L1_mrna_build.1	1925 bp	10 exons	#115		
exon	1169	1319				
exon	1572	1601				
exon	2036	2237				
exon	2789	2845				
exon	3606	3756				
exon	4517	4638				
exon	5436	5559				
exon	6069	6251				
exon	6844	6960				
exon	7136	7923				
CDS	CHI3L1_cds.4	69 bp	3 exons	#109		
exon	1295	1319				
exon	1572	1601				
exon	2789	2802				
Allele	GB:Y08376_1	112	311	311	G>T	
	source	isSNP	SNP00071934			
	consequence	CHI3L1_cds.1	106	Intron		
	consequence	CHI3L1_cds.2	107	Intron		
	consequence	CHI3L1_cds.3	108	Intron		
	consequence	CHI3L1_cds.4	109	3'		
Allele	GB:Y08376_1	112	438	438	A>G	
	source	isSNP	SNP00008252			
	consequence	CHI3L1_cds.1	106	Missense	145-145	R>G
	consequence	CHI3L1_cds.2	107	Missense	145-145	R>G
	consequence	CHI3L1_cds.3	108	Missense	145-145	R>G
	consequence	CHI3L1_cds.4	109	3'		
Allele	GB:Y08377_1	113	355	355	G>T	
	source	isSNP	SNP00022932			
	consequence	CHI3L1_cds.1	106	Missense	174-174	L>I
	consequence	CHI3L1_cds.2	107	Missense	174-174	L>I
	consequence	CHI3L1_cds.3	108	Missense	174-174	L>I
	consequence	CHI3L1_cds.4	109	3'		
Allele	GB:Y08378_1	114	506	506	A>G	
	source	isSNP	SNP00005491			
	consequence	CHI3L1_cds.1	106	Intron		
	consequence	CHI3L1_cds.2	107	Intron		
	consequence	CHI3L1_cds.3	108	Intron		
	consequence	CHI3L1_cds.4	109	3'		
Allele	GB:Y08378_1	114	535	535	A>G	
	source	isSNP	SNP00005492			
	consequence	CHI3L1_cds.1	106	Intron		
	consequence	CHI3L1_cds.2	107	Intron		
	consequence	CHI3L1_cds.3	108	Intron		
	consequence	CHI3L1_cds.4	109	3'		
Allele	GB:Y08378_1	114	641	641	A>G	
	source	isSNP	SNP00028111			
	consequence	CHI3L1_cds.1	106	Intron		
	consequence	CHI3L1_cds.2	107	Intron		
	consequence	CHI3L1_cds.3	108	Intron		
	consequence	CHI3L1_cds.4	109	3'		
Allele	GB:Y08378_1	114	1560	1560	A>G	
	source	isSNP	SNP00028112			
	consequence	CHI3L1_cds.1	106	Intron		
	consequence	CHI3L1_cds.2	107	Intron		

TABLE 1 (Cont.)

	consequence	CHI3L1_cds.3	108	Intron		
	consequence	CHI3L1_cds.4	109	3'		
Allele	GB:Y08378_1	114 2163 2163	A>G			
	source	isSNP SNP00052666				
	consequence	CHI3L1_cds.1	106	3'		
	consequence	CHI3L1_cds.2	107	Silent	338-338	H
	consequence	CHI3L1_cds.3	108	3'		
	consequence	CHI3L1_cds.4	109	3'		
Allele	GB:Y08378_1	114 2205 2205	A>G			
	source	isSNP SNP00072805				
	consequence	CHI3L1_cds.1	106	3'		
	consequence	CHI3L1_cds.2	107	Silent	352-352	A
	consequence	CHI3L1_cds.3	108	3'		
	consequence	CHI3L1_cds.4	109	3'		
Allele	GB:Y08378_1	114 2602 2602	A>G			
	source	isSNP SNP00076686				
	consequence	CHI3L1_cds.1	106	3'		
	consequence	CHI3L1_cds.2	107	3'		
	consequence	CHI3L1_cds.3	108	3'		
	consequence	CHI3L1_cds.4	109	3'		
GIF CHI3L1-genomic-fwd.gif						

## CHI3L2

Full name : chitinase 3-like 2

Link : CHI3L2\_link\_cdna

Subsequence	GB:HSU58514	1	1434	#117		
CDS	GB:HSU58514.1	1173 bp		#118		
ORF	37	1209				
Allele	GB:HSU58514	117 412 412	A>G			
	source	isSNP SNP00021152				
	consequence	GB:HSU58514.1	118	Missense	126-126	N>D
Allele	GB:HSU58514	117 581 581	A>G			
	source	isSNP SNP00021153				
	consequence	GB:HSU58514.1	118	Missense	182-182	A>V
Allele	GB:HSU58514	117 972 972	A>G			
	source	isSNP SNP00115597				
	consequence	GB:HSU58514.1	118	Silent	312-312	K
Allele	GB:HSU58514	117 1204 1204	A>G			
	source	isSNP SNP00068229				
	consequence	GB:HSU58514.1	118	Silent	390-390	L

GIF CHI3L2-cdna-fwd.gif

Link : CHI3L2\_alt\_link\_cdna

Subsequence	GB:U58515_1	1	1500	#119		
CDS	GB:U58515_1.1	1275 bp		#120		
ORF	1	1275				
Allele	GB:U58515_1	119 478 478	A>G			
	source	isSNP SNP00021152				
	consequence	GB:U58515_1.1	120	Missense	160-160	N>D
Allele	GB:U58515_1	119 647 647	A>G			
	source	isSNP SNP00021153				
	consequence	GB:U58515_1.1	120	Missense	216-216	A>V
Allele	GB:U58515_1	119 1038 1038	A>G			
	source	isSNP SNP00115597				

TABLE 1 (Cont.)

	consequence	GB:U58515_1.1	120	Silent	346-346	K
Allele	GB:U58515_1	119 1270 1270	A>G			
	source	isSNP SNP00068229				
	consequence	GB:U58515_1.1	120	Silent	424-424	L

GIF CHI3L2-cdna-fwd.gif

## CILP

Full name : cartilage intermediate layer protein

Link : CILP\_link\_cdna

Subsequence	GB:AF035408	1	4175	#121		
CDS	GB:AF035408.1	3555 bp		#122		
ORF	130	3684				
Allele	GB:AF035408	121 430 430	A>G			
	source	isSNP SNP00123071				
	consequence	GB:AF035408.1	122	Missense	101-101	P>S
Allele	GB:AF035408	121 1677 1677	A>G			
	source	isSNP SNP00123072				
	consequence	GB:AF035408.1	122	Silent	516-516	R
Allele	GB:AF035408	121 3066 3066	A>G			
	source	isSNP SNP00020276				
	consequence	GB:AF035408.1	122	Silent	979-979	R
Allele	GB:AF035408	121 3263 3263	A>G			
	source	isSNP SNP00123073				
	consequence	GB:AF035408.1	122	Missense	1045-1045	Y>C
Allele	GB:AF035408	121 3625 3625	A>G			
	source	isSNP SNP00055164				
	consequence	GB:AF035408.1	122	Missense	1166-1166	S>G

GIF CILP-cdna-fwd.gif

Link : CILP\_link\_genomic

Subsequence	CILP_cds.1	3606	16639	#123		
Subsequence	GB:AB022430_1	1	19486	#124		
Subsequence	CILP_mrna_build.1	1911	17130	#125		
CDS	CILP_cds.1	3555 bp	8 exons	#123		
exon	3606	3666				
exon	5599	5691				
exon	6312	6581				
exon	7897	8076				
exon	8781	9095				
exon	9893	10001				
exon	11336	11493				
exon	14271	16639				
mRNA	CILP_mrna_build.1	4175 bp	9 exons	#125		
exon	1911	1933				
exon	3500	3666				
exon	5599	5691				
exon	6312	6581				
exon	7897	8076				
exon	8781	9095				
exon	9893	10001				
exon	11336	11493				
exon	14271	17130				
Allele	GB:AB022430_1	124	3567 3567	G>T		
	source	wetSNP	GB:AB022430_1.v3567	A>C		

TABLE 1 (Cont.)

	consequence	CILP_cds.1	123	5'		
Allele	GB:AB022430_1	124	6458	6458	A>G	
	source	isSNP	SNP00123071			
	consequence	CILP_cds.1	123	Missense	101-101	P>S
Allele	GB:AB022430_1	124	9874	9874	A>G	
	source	wetSNP	GB:AB022430_1.v9874.C>T			
	consequence	CILP_cds.1	123	Intron		
Allele	GB:AB022430_1	124	9881	9881	A>G	
	source	wetSNP	GB:AB022430_1.v9881.C>T			
	consequence	CILP_cds.1	123	Intron		
Allele	GB:AB022430_1	124	11286	11286	A>T	
	source	wetSNP	GB:AB022430_1.v11286.T>A			
	consequence	CILP_cds.1	123	Intron		
Allele	GB:AB022430_1	124	11491	11491	A>G	
	source	wetSNP	GB:AB022430_1.v11491.C>T			
	consequence	CILP_cds.1	123	Missense	395-395	T>I
Allele	GB:AB022430_1	124	14421	14421	C>G	
	source	wetSNP	GB:AB022430_1.v14421.G>C			
	consequence	CILP_cds.1	123	Missense	446-446	R>T
Allele	GB:AB022430_1	124	14542	14542	A>G	
	source	wetSNP	GB:AB022430_1.v14542.G>A			
	consequence	CILP_cds.1	123	Silent	486-486	T
Allele	GB:AB022430_1	124	14632	14632	A>G	
	source	isSNP	SNP00123072			
	consequence	CILP_cds.1	123	Silent	516-516	R
Allele	GB:AB022430_1	124	15116	15116	A>G	
	source	wetSNP	GB:AB022430_1.v15116.G>A			
	consequence	CILP_cds.1	123	Missense	678-678	V>M
Allele	GB:AB022430_1	124	15670	15670	A>G	
	source	wetSNP	GB:AB022430_1.v15670.G>A			
	consequence	CILP_cds.1	123	Silent	862-862	T
Allele	GB:AB022430_1	124	16021	16021	A>G	
	source	isSNP	SNP00020276			
	consequence	CILP_cds.1	123	Silent	979-979	R
Allele	GB:AB022430_1	124	16218	16218	A>G	
	source	isSNP	SNP00123073			
	consequence	CILP_cds.1	123	Missense	1045-1045	Y>C
Allele	GB:AB022430_1	124	16580	16580	A>G	
	source	isSNP	SNP00055164			
	source	wetSNP	GB:AB022430_1.v16580.A>G			
	consequence	CILP_cds.1	123	Missense	1166-1166	S>G
GIF CILP-genomic-fwd.gif						

## COL10A1

Full name : collagen, type X, alpha 1

Link : COL10A1\_link\_cdna

Subsequence	GB:X60382_1	1	3226	#126		
CDS	GB:X60382_1.2	2043 bp		#127		
ORF	16	2058				
Allele	GB:X60382_1	126	95	95	A>G	
	source	isSNP	SNP00034488			
	consequence	GB:X60382_1.2	127	Missense	27-27	T>M
Allele	GB:X60382_1	126	2294	2294	G>T	
			167			

TABLE 1 (Cont.)

source	isSNP	SNP00113056		
consequence	GB:X60382_1.2	127	3'	
GIF COL10A1-cdna-fwd.gif				
COL11A2				
Full name : collagen, type XI, alpha 2				
Link : FL_3421462_link_genomic				
Subsequence	GB:AL031228_1	1	175737	#128
Subsequence	COL11A2_cds.1	93988	122550	#129
Subsequence	COL11A2_cds.2	93988	122550	#130
Subsequence	COL11A2_cds.3	93988	122550	#131
Subsequence	COL11A2_cds.4	93988	122550	#132
Subsequence	COL11A2_cds.5	93988	122550	#133
Subsequence	COL11A2_cds.6	93988	122550	#134
Subsequence	COL11A2_cds.7	93988	122550	#135
Subsequence	COL11A2_cds.8	93988	122550	#136
Subsequence	COL11A2_mrna_build.1	93988	122834	#137
Subsequence	COL11A2_mrna_build.2	93988	122834	#138
Subsequence	GB:AL031228_1.20	93762	123536	#139
Subsequence	GB:AL031228_1.21	93988	122550	#140
Subsequence	COL11A2_mrna_build.3	93769	125002	#141
mRNA	GB:AL031228_1.20	6423 bp	66 exons	#139
exon	93762	94069		
exon	96759	96908		
exon	97040	97250		
exon	97704	97866		
exon	99410	99601		
exon	100450	100527		
exon	101174	101236		
exon	101904	102083		
exon	105058	105117		
exon	105223	105264		
exon	105498	105560		
exon	105896	105970		
exon	106423	106509		
exon	106741	106797		
exon	106944	106997		
exon	107102	107155		
exon	107255	107308		
exon	107496	107549		
exon	107740	107793		
exon	107876	107920		
exon	108043	108096		
exon	108522	108566		
exon	108763	108816		
exon	109003	109047		
exon	109183	109236		
exon	109463	109507		
exon	109742	109795		
exon	109925	109969		
exon	110159	110212		
exon	110547	110654		
exon	111648	111701		

TABLE 1 (Cont.)

exon	112010	112063			
exon	112173	112217			
exon	112302	112355			
exon	112483	112527			
exon	112673	112726			
exon	112827	112880			
exon	113115	113168			
exon	113591	113698			
exon	113850	113939			
exon	114125	114178			
exon	114408	114515			
exon	114654	114761			
exon	114904	114957			
exon	115061	115114			
exon	115311	115418			
exon	115618	115671			
exon	115849	115902			
exon	116128	116181			
exon	116344	116397			
exon	116738	116845			
exon	117220	117273			
exon	117469	117522			
exon	117656	117709			
exon	118376	118429			
exon	118695	118802			
exon	118911	118964			
exon	119105	119158			
exon	119401	119508			
exon	119662	119715			
exon	120022	120057			
exon	120244	120297			
exon	120412	120679			
exon	121264	121376			
exon	121755	121961			
exon	122410	123536			
mRNA	COL11A2_mrna_build.3	6780 bp	66 exons	#141	
exon	93769 94341				
exon	96759 96908				
exon	97040 97250				
exon	97704 97866				
exon	99410 99601				
exon	101174	101236			
exon	101904	102083			
exon	105058	105117			
exon	105223	105264			
exon	105498	105560			
exon	105896	105970			
exon	106423	106509			
exon	106741	106797			
exon	106944	106997			
exon	107102	107155			
exon	107255	107308			
exon	107496	107549			
exon	107740	107793			
exon	107876	107920			

TABLE 1 (Cont.)

exon	108043	108096		
exon	108522	108566		
exon	108763	108816		
exon	109003	109047		
exon	109183	109236		
exon	109463	109507		
exon	109742	109795		
exon	109925	109969		
exon	110159	110212		
exon	110547	110654		
exon	111648	111701		
exon	112010	112063		
exon	112173	112217		
exon	112302	112355		
exon	112483	112577		
exon	112673	112726		
exon	112827	112880		
exon	113115	113168		
exon	113591	113698		
exon	113850	113939		
exon	114125	114178		
exon	114408	114515		
exon	114654	114761		
exon	114904	114957		
exon	115061	115114		
exon	115311	115418		
exon	115618	115671		
exon	115849	115902		
exon	116128	116196		
exon	116344	116397		
exon	116738	116845		
exon	117220	117273		
exon	117469	117522		
exon	117656	117709		
exon	118376	118429		
exon	118695	118802		
exon	118911	118964		
exon	119105	119158		
exon	119401	119508		
exon	120022	120057		
exon	120244	120297		
exon	120412	120679		
exon	121264	121376		
exon	121755	121961		
exon	122183	122332		
exon	122410	123530		
exon	124988	125002		
CDS	COL11A2_cds.6	5157 bp	65 exons	#134
exon	93988	94069		
exon	96759	96908		
exon	97040	97250		
exon	97704	97866		
exon	99410	99601		
exon	100450	100527		
exon	101174	101236		



TABLE 1 (Cont.)

exon	101904	102083
exon	105058	105117
exon	105223	105264
exon	105498	105560
exon	105896	105970
exon	106423	106509
exon	106741	106797
exon	106944	106997
exon	107102	107155
exon	107255	107308
exon	107496	107549
exon	107740	107793
exon	107876	107920
exon	108043	108096
exon	108522	108566
exon	108763	108816
exon	109003	109047
exon	109183	109236
exon	109463	109507
exon	109742	109795
exon	109925	109969
exon	110159	110212
exon	110547	110654
exon	111648	111701
exon	112010	112063
exon	112173	112217
exon	112302	112355
exon	112483	112527
exon	112673	112726
exon	112827	112880
exon	113115	113168
exon	113591	113698
exon	113850	113939
exon	114125	114178
exon	114408	114515
exon	114654	114761
exon	114904	114957
exon	115061	115114
exon	115311	115418
exon	115618	115671
exon	115849	115902
exon	116128	116181
exon	116344	116397
exon	116738	116845
exon	117220	117273
exon	117469	117522
exon	117656	117709
exon	118376	118429
exon	118695	118802
exon	118911	118964
exon	119105	119158
exon	119401	119508
exon	120022	120057
exon	120244	120297
exon	120412	120679

TABLE 1 (Cont.)

	exon	121264	121376		
	exon	121755	121961		
	exon	122410	122550		
CDS	GB:AL031228_1.21	5211 bp	66 exons	#140	
	exon	93988 94069			
	exon	96759 96908			
	exon	97040 97250			
	exon	97704 97866			
	exon	99410 99601			
	exon	100450	100527		
	exon	101174	101236		
	exon	101904	102083		
	exon	105058	105117		
	exon	105223	105264		
	exon	105498	105560		
	exon	105896	105970		
	exon	106423	106509		
	exon	106741	106797		
	exon	106944	106997		
	exon	107102	107155		
	exon	107255	107308		
	exon	107496	107549		
	exon	107740	107793		
	exon	107876	107920		
	exon	108043	108096		
	exon	108522	108566		
	exon	108763	108816		
	exon	109003	109047		
	exon	109183	109236		
	exon	109463	109507		
	exon	109742	109795		
	exon	109925	109969		
	exon	110159	110212		
	exon	110547	110654		
	exon	111648	111701		
	exon	112010	112063		
	exon	112173	112217		
	exon	112302	112355		
	exon	112483	112527		
	exon	112673	112726		
	exon	112827	112880		
	exon	113115	113168		
	exon	113591	113698		
	exon	113850	113939		
	exon	114125	114178		
	exon	114408	114515		
	exon	114654	114761		
	exon	114904	114957		
	exon	115061	115114		
	exon	115311	115418		
	exon	115618	115671		
	exon	115849	115902		
	exon	116128	116181		
	exon	116344	116397		
	exon	116738	116845		

TABLE 1 (Cont.)

exon	117220	117273		
exon	117469	117522		
exon	117656	117709		
exon	118376	118429		
exon	118695	118802		
exon	118911	118964		
exon	119105	119158		
exon	119401	119508		
exon	119662	119715		
exon	120022	120057		
exon	120244	120297		
exon	120412	120679		
exon	121264	121376		
exon	121755	121961		
exon	122410	122550		
CDS	COL11A2_cds.7	5049 bp	64 exons	#135
exon	93988 94069			
exon	96759 96908			
exon	97040 97250			
exon	97704 97866			
exon	99410 99601			
exon	101174	101236		
exon	105058	105117		
exon	105223	105264		
exon	105498	105560		
exon	105896	105970		
exon	106423	106509		
exon	106741	106797		
exon	106944	106997		
exon	107102	107155		
exon	107255	107308		
exon	107496	107549		
exon	107740	107793		
exon	107876	107920		
exon	108043	108096		
exon	108522	108566		
exon	108763	108816		
exon	109003	109047		
exon	109183	109236		
exon	109463	109507		
exon	109742	109795		
exon	109925	109969		
exon	110159	110212		
exon	110547	110654		
exon	111648	111701		
exon	112010	112063		
exon	112173	112217		
exon	112302	112355		
exon	112483	112527		
exon	112673	112726		
exon	112827	112880		
exon	113115	113168		
exon	113591	113698		
exon	113850	113939		
exon	114125	114178		

TABLE 1 (Cont.)

exon	114408	114515		
exon	114654	114761		
exon	114904	114957		
exon	115061	115114		
exon	115311	115418		
exon	115618	115671		
exon	115849	115902		
exon	116128	116181		
exon	116344	116397		
exon	116738	116845		
exon	117220	117273		
exon	117469	117522		
exon	117656	117709		
exon	118376	118429		
exon	118695	118802		
exon	118911	118964		
exon	119105	119158		
exon	119401	119508		
exon	120022	120057		
exon	120244	120297		
exon	120412	120679		
exon	121264	121376		
exon	121755	121961		
exon	122183	122332		
exon	122410	122550		
CDS	COL11A2_cds.8	4986 bp	63 exons	#136
exon	93988 94069			
exon	96759 96908			
exon	97040 97250			
exon	97704 97866			
exon	99410 99601			
exon	105058	105117		
exon	105223	105264		
exon	105498	105560		
exon	105896	105970		
exon	106423	106509		
exon	106741	106797		
exon	106944	106997		
exon	107102	107155		
exon	107255	107308		
exon	107496	107549		
exon	107740	107793		
exon	107876	107920		
exon	108043	108096		
exon	108522	108566		
exon	108763	108816		
exon	109003	109047		
exon	109183	109236		
exon	109463	109507		
exon	109742	109795		
exon	109925	109969		
exon	110159	110212		
exon	110547	110654		
exon	111648	111701		
exon	112010	112063		

TABLE 1 (Cont.)

exon	112173	112217		
exon	112302	112355		
exon	112483	112527		
exon	112673	112726		
exon	112827	112880		
exon	113115	113168		
exon	113591	113698		
exon	113850	113939		
exon	114125	114178		
exon	114408	114515		
exon	114654	114761		
exon	114904	114957		
exon	115061	115114		
exon	115311	115418		
exon	115618	115671		
exon	115849	115902		
exon	116128	116181		
exon	116344	116397		
exon	116738	116845		
exon	117220	117273		
exon	117469	117522		
exon	117656	117709		
exon	118376	118429		
exon	118695	118802		
exon	118911	118964		
exon	119105	119158		
exon	119401	119508		
exon	120022	120057		
exon	120244	120297		
exon	120412	120679		
exon	121264	121376		
exon	121755	121961		
exon	122183	122332		
exon	122410	122550		
CDS	COL11A2_cds.1	4890 bp	63 exons	#129
exon	93988	94069		
exon	96759	96908		
exon	97040	97250		
exon	97704	97866		
exon	99410	99601		
exon	105058	105117		
exon	105223	105264		
exon	105498	105560		
exon	105896	105970		
exon	106423	106509		
exon	106741	106797		
exon	106944	106997		
exon	107102	107155		
exon	107255	107308		
exon	107496	107549		
exon	107740	107793		
exon	107876	107920		
exon	108043	108096		
exon	108522	108566		
exon	108763	108816		

TABLE 1 (Cont.)

exon	109003	109047		
exon	109183	109236		
exon	109463	109507		
exon	109742	109795		
exon	109925	109969		
exon	110159	110212		
exon	110547	110654		
exon	111648	111701		
exon	112010	112063		
exon	112173	112217		
exon	112302	112355		
exon	112483	112527		
exon	112673	112726		
exon	112827	112880		
exon	113115	113168		
exon	113591	113698		
exon	113850	113939		
exon	114125	114178		
exon	114408	114515		
exon	114654	114761		
exon	114904	114957		
exon	115061	115114		
exon	115311	115418		
exon	115618	115671		
exon	115849	115902		
exon	116128	116181		
exon	116344	116397		
exon	116738	116845		
exon	117220	117273		
exon	117469	117522		
exon	117656	117709		
exon	118376	118429		
exon	118695	118802		
exon	118911	118964		
exon	119105	119158		
exon	119401	119508		
exon	119662	119715		
exon	120022	120057		
exon	120244	120297		
exon	120412	120679		
exon	121264	121376		
exon	121755	121961		
exon	122410	122550		
CDS	COL11A2_cds.2	4953 bp	64 exons	#130
exon	93988 94069			
exon	96759 96908			
exon	97040 97250			
exon	97704 97866			
exon	99410 99601			
exon	101174	101236		
exon	105058	105117		
exon	105223	105264		
exon	105498	105560		
exon	105896	105970		
exon	106423	106509		

TABLE 1 (Cont.)

exon	106741	106797		
exon	106944	106997		
exon	107102	107155		
exon	107255	107308		
exon	107496	107549		
exon	107740	107793		
exon	107876	107920		
exon	108043	108096		
exon	108522	108566		
exon	108763	108816		
exon	109003	109047		
exon	109183	109236		
exon	109463	109507		
exon	109742	109795		
exon	109925	109969		
exon	110159	110212		
exon	110547	110654		
exon	111648	111701		
exon	112010	112063		
exon	112173	112217		
exon	112302	112355		
exon	112483	112527		
exon	112673	112726		
exon	112827	112880		
exon	113115	113168		
exon	113591	113698		
exon	113850	113939		
exon	114125	114178		
exon	114408	114515		
exon	114654	114761		
exon	114904	114957		
exon	115061	115114		
exon	115311	115418		
exon	115618	115671		
exon	115849	115902		
exon	116128	116181		
exon	116344	116397		
exon	116738	116845		
exon	117220	117273		
exon	117469	117522		
exon	117656	117709		
exon	118376	118429		
exon	118695	118802		
exon	118911	118964		
exon	119105	119158		
exon	119401	119508		
exon	119662	119715		
exon	120022	120057		
exon	120244	120297		
exon	120412	120679		
exon	121264	121376		
exon	121755	121961		
exon	122410	122550		
CDS	COL11A2_cds.3	5307 bp	66 exons	#131
exon	93988 94069			

TABLE 1 (Cont.)

exon	96759	96908
exon	97040	97250
exon	97704	97866
exon	99410	99601
exon	100450	100527
exon	101174	101236
exon	101904	102083
exon	105058	105117
exon	105223	105264
exon	105498	105560
exon	105896	105970
exon	106423	106509
exon	106741	106797
exon	106944	106997
exon	107102	107155
exon	107255	107308
exon	107496	107549
exon	107740	107793
exon	107876	107920
exon	108043	108096
exon	108522	108566
exon	108763	108816
exon	109003	109047
exon	109183	109236
exon	109463	109507
exon	109742	109795
exon	109925	109969
exon	110159	110212
exon	110547	110654
exon	111648	111701
exon	112010	112063
exon	112173	112217
exon	112302	112355
exon	112483	112527
exon	112673	112726
exon	112827	112880
exon	113115	113168
exon	113591	113698
exon	113850	113939
exon	114125	114178
exon	114408	114515
exon	114654	114761
exon	114904	114957
exon	115061	115114
exon	115311	115418
exon	115618	115671
exon	115849	115902
exon	116128	116181
exon	116344	116397
exon	116738	116845
exon	117220	117273
exon	117469	117522
exon	117656	117709
exon	118376	118429
exon	118695	118802



TABLE 1 (Cont.)

exon	118911	118964			
exon	119105	119158			
exon	119401	119508			
exon	120022	120057			
exon	120244	120297			
exon	120412	120679			
exon	121264	121376			
exon	121755	121961			
exon	122183	122332			
exon	122410	122550			
mRNA	COL11A2_mrna_build.1		5174 bp	63 exons	#137
exon	93988	94069			
exon	96759	96908			
exon	97040	97250			
exon	97704	97866			
exon	99410	99601			
exon	105058	105117			
exon	105223	105264			
exon	105498	105560			
exon	105896	105970			
exon	106423	106509			
exon	106741	106797			
exon	106944	106997			
exon	107102	107155			
exon	107255	107308			
exon	107496	107549			
exon	107740	107793			
exon	107876	107920			
exon	108043	108096			
exon	108522	108566			
exon	108763	108816			
exon	109003	109047			
exon	109183	109236			
exon	109463	109507			
exon	109742	109795			
exon	109925	109969			
exon	110159	110212			
exon	110547	110654			
exon	111648	111701			
exon	112010	112063			
exon	112173	112217			
exon	112302	112355			
exon	112483	112527			
exon	112673	112726			
exon	112827	112880			
exon	113115	113168			
exon	113591	113698			
exon	113850	113939			
exon	114125	114178			
exon	114408	114515			
exon	114654	114761			
exon	114904	114957			
exon	115061	115114			
exon	115311	115418			
exon	115618	115671			

TABLE 1 (Cont.)

exon	115849	115902		
exon	116128	116181		
exon	116344	116397		
exon	116738	116845		
exon	117220	117273		
exon	117469	117522		
exon	117656	117709		
exon	118376	118429		
exon	118695	118802		
exon	118911	118964		
exon	119105	119158		
exon	119401	119508		
exon	119662	119715		
exon	120022	120057		
exon	120244	120297		
exon	120412	120679		
exon	121264	121376		
exon	121755	121961		
exon	122410	122834		
CDS	COL11A2_cds.4	4836 bp	62 exons	#132
exon	93988	94069		
exon	96759	96908		
exon	97040	97250		
exon	97704	97866		
exon	99410	99601		
exon	105058	105117		
exon	105223	105264		
exon	105498	105560		
exon	105896	105970		
exon	106423	106509		
exon	106741	106797		
exon	106944	106997		
exon	107102	107155		
exon	107255	107308		
exon	107496	107549		
exon	107740	107793		
exon	107876	107920		
exon	108043	108096		
exon	108522	108566		
exon	108763	108816		
exon	109003	109047		
exon	109183	109236		
exon	109463	109507		
exon	109742	109795		
exon	109925	109969		
exon	110159	110212		
exon	110547	110654		
exon	111648	111701		
exon	112010	112063		
exon	112173	112217		
exon	112302	112355		
exon	112483	112527		
exon	112673	112726		
exon	112827	112880		
exon	113115	113168		

TABLE 1 (Cont.)

exon	113591	113698			
exon	113850	113939			
exon	114125	114178			
exon	114408	114515			
exon	114654	114761			
exon	114904	114957			
exon	115061	115114			
exon	115311	115418			
exon	115618	115671			
exon	115849	115902			
exon	116128	116181			
exon	116344	116397			
exon	116738	116845			
exon	117220	117273			
exon	117469	117522			
exon	117656	117709			
exon	118376	118429			
exon	118695	118802			
exon	118911	118964			
exon	119105	119158			
exon	119401	119508			
exon	120022	120057			
exon	120244	120297			
exon	120412	120679			
exon	121264	121376			
exon	121755	121961			
exon	122410	122550			
mRNA	COL11A2_mrna_build.2		5237 bp	64 exons	#138
exon	93988	94069			
exon	96759	96908			
exon	97040	97250			
exon	97704	97866			
exon	99410	99601			
exon	101174	101236			
exon	105058	105117			
exon	105223	105264			
exon	105498	105560			
exon	105896	105970			
exon	106423	106509			
exon	106741	106797			
exon	106944	106997			
exon	107102	107155			
exon	107255	107308			
exon	107496	107549			
exon	107740	107793			
exon	107876	107920			
exon	108043	108096			
exon	108522	108566			
exon	108763	108816			
exon	109003	109047			
exon	109183	109236			
exon	109463	109507			
exon	109742	109795			
exon	109925	109969			
exon	110159	110212			

TABLE 1 (Cont.)

exon	110547	110654		
exon	111648	111701		
exon	112010	112063		
exon	112173	112217		
exon	112302	112355		
exon	112483	112527		
exon	112673	112726		
exon	112827	112880		
exon	113115	113168		
exon	113591	113698		
exon	113850	113939		
exon	114125	114178		
exon	114408	114515		
exon	114654	114761		
exon	114904	114957		
exon	115061	115114		
exon	115311	115418		
exon	115618	115671		
exon	115849	115902		
exon	116128	116181		
exon	116344	116397		
exon	116738	116845		
exon	117220	117273		
exon	117469	117522		
exon	117656	117709		
exon	118376	118429		
exon	118695	118802		
exon	118911	118964		
exon	119105	119158		
exon	119401	119508		
exon	119662	119715		
exon	120022	120057		
exon	120244	120297		
exon	120412	120679		
exon	121264	121376		
exon	121755	121961		
exon	122410	122834		
CDS	COL11A2_cds.5	4899 bp	63 exons	#133
exon	93988	94069		
exon	96759	96908		
exon	97040	97250		
exon	97704	97866		
exon	99410	99601		
exon	101174	101236		
exon	105058	105117		
exon	105223	105264		
exon	105498	105560		
exon	105896	105970		
exon	106423	106509		
exon	106741	106797		
exon	106944	106997		
exon	107102	107155		
exon	107255	107308		
exon	107496	107549		
exon	107740	107793		

TABLE 1 (Cont.)

exon	107876	107920			
exon	108043	108096			
exon	108522	108566			
exon	108763	108816			
exon	109003	109047			
exon	109183	109236			
exon	109463	109507			
exon	109742	109795			
exon	109925	109969			
exon	110159	110212			
exon	110547	110654			
exon	111648	111701			
exon	112010	112063			
exon	112173	112217			
exon	112302	112355			
exon	112483	112527			
exon	112673	112726			
exon	112827	112880			
exon	113115	113168			
exon	113591	113698			
exon	113850	113939			
exon	114125	114178			
exon	114408	114515			
exon	114654	114761			
exon	114904	114957			
exon	115061	115114			
exon	115311	115418			
exon	115618	115671			
exon	115849	115902			
exon	116128	116181			
exon	116344	116397			
exon	116738	116845			
exon	117220	117273			
exon	117469	117522			
exon	117656	117709			
exon	118376	118429			
exon	118695	118802			
exon	118911	118964			
exon	119105	119158			
exon	119401	119508			
exon	120022	120057			
exon	120244	120297			
exon	120412	120679			
exon	121264	121376			
exon	121755	121961			
exon	122410	122550			
Allele	GB:AL031228_1	128	122970	122970	A>G
	source	isSNP	SNP00027609		
	consequence	COL11A2_cds.6	134	3'	
	consequence	GB:AL031228_1.21	140	3'	
	consequence	COL11A2_cds.7	135	3'	
	consequence	COL11A2_cds.8	136	3'	
	consequence	COL11A2_cds.1	129	3'	
	consequence	COL11A2_cds.2	130	3'	
	consequence	COL11A2_cds.3	131	3'	

TABLE 1 (Cont.)

consequence COL11A2\_cds.4 132 3'  
 consequence COL11A2\_cds.5 133 3'  
 GIF COL11A2-genomic-fwd.gif

## COL9A2

Full name : collagen, type IX, alpha 2

Link : FL\_3482334\_link\_cdna

Subsequence	FN:3482334CB1	1	2864	#142		
CDS	FN:3482334CB1.1	2079 bp		#143		
ORF	99	2177				
Allele	FN:3482334CB1	142 1087	1087	A>G		
	source	isSNP SNP00032502				
	consequence	FN:3482334CB1.1	143	Missense	330-330	Q>R
Allele	FN:3482334CB1	142 1113	1113	C>G		
	source	isSNP SNP00107342				
	consequence	FN:3482334CB1.1	143	Missense	339-339	L>V
Allele	FN:3482334CB1	142 1301	1301	A>G		
	source	isSNP SNP00107343				
	consequence	FN:3482334CB1.1	143	Silent	401-401	G
Allele	FN:3482334CB1	142 1345	1345	C>G		
	source	isSNP SNP00107344				
	consequence	FN:3482334CB1.1	143	Missense	416-416	G>A
Allele	FN:3482334CB1	142 2211	2211	A>G		
	source	isSNP SNP00067542				
	consequence	FN:3482334CB1.1	143	3'		
Allele	FN:3482334CB1	142 2317	2317	A>G		
	source	isSNP SNP00032503				
	consequence	FN:3482334CB1.1	143	3'		

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Link : FL\_1651412\_link\_cdna

Subsequence	FN:1651412CB1	1	2869	#144		
CDS	FN:1651412CB1.1	2067 bp		#145		
ORF	68	2134				
Allele	FN:1651412CB1	144 1044	1044	A>G		
	source	isSNP SNP00032502				
	consequence	FN:1651412CB1.1	145	Missense	326-326	R>Q
Allele	FN:1651412CB1	144 1070	1070	C>G		
	source	isSNP SNP00107342				
	consequence	FN:1651412CB1.1	145	Missense	335-335	L>V
Allele	FN:1651412CB1	144 1258	1258	A>G		
	source	isSNP SNP00107343				
	consequence	FN:1651412CB1.1	145	Silent	397-397	G
Allele	FN:1651412CB1	144 1302	1302	C>G		
	source	isSNP SNP00107344				
	consequence	FN:1651412CB1.1	145	Missense	412-412	G>A
Allele	FN:1651412CB1	144 2168	2168	A>G		
	source	isSNP SNP00067542				
	consequence	FN:1651412CB1.1	145	3'		
Allele	FN:1651412CB1	144 2274	2274	A>G		
	source	isSNP SNP00032503				
	consequence	FN:1651412CB1.1	145	3'		

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Link : FL\_1651412\_link\_genomic

TABLE 1 (Cont.)

Subsequence	GB:AF019406	1	17606	#146	
Subsequence	GB:AF019406_1651412CD1	1115	17091	#147	
Subsequence	GB:AF019406_3482334CD1	1115	17091	#148	
Subsequence	FL_1651412_mrna_build.1	1048	17606	#149	
Subsequence	FL_3482334_mrna_build.1	1017	17606	#150	
mRNA	FL_1651412_mrna_build.1	2649 bp	32 exons	#149	
exon	1048	1189			
exon	2635	2709			
exon	3905	3940			
exon	4025	4087			
exon	5507	5560			
exon	5682	5717			
exon	5811	5834			
exon	6178	6231			
exon	6573	6626			
exon	6741	6788			
exon	7002	7058			
exon	7142	7195			
exon	7521	7574			
exon	7971	8024			
exon	8124	8177			
exon	8297	8350			
exon	10041	10094			
exon	10530	10583			
exon	10787	10840			
exon	12101	12145			
exon	12519	12572			
exon	13436	13489			
exon	13754	13807			
exon	13892	13963			
exon	14184	14219			
exon	14311	14355			
exon	14440	14472			
exon	14603	14749			
exon	15093	15147			
exon	15467	15655			
exon	16387	16464			
exon	16895	17606			
CDS	GB:AF019406_3482334CD1	2079 bp	32 exons	#148	
exon	1115	1189			
exon	2635	2709			
exon	3905	3940			
exon	4025	4087			
exon	5507	5560			
exon	5682	5717			
exon	5811	5834			
exon	6178	6231			
exon	6573	6626			
exon	6741	6800			
exon	7002	7058			
exon	7142	7195			
exon	7521	7574			
exon	7971	8024			
exon	8124	8177			
exon	8297	8350			

TABLE 1 (Cont.)

exon	10041	10094			
exon	10530	10583			
exon	10787	10840			
exon	12101	12145			
exon	12519	12572			
exon	13436	13489			
exon	13754	13807			
exon	13892	13963			
exon	14184	14219			
exon	14311	14355			
exon	14440	14472			
exon	14603	14749			
exon	15093	15147			
exon	15467	15655			
exon	16387	16464			
exon	16895	17091			
mRNA	FL_3482334_mrna_build.1	2692 bp	32 exons	#150	
exon	1017	1189			
exon	2635	2709			
exon	3905	3940			
exon	4025	4087			
exon	5507	5560			
exon	5682	5717			
exon	5811	5834			
exon	6178	6231			
exon	6573	6626			
exon	6741	6800			
exon	7002	7058			
exon	7142	7195			
exon	7521	7574			
exon	7971	8024			
exon	8124	8177			
exon	8297	8350			
exon	10041	10094			
exon	10530	10583			
exon	10787	10840			
exon	12101	12145			
exon	12519	12572			
exon	13436	13489			
exon	13754	13807			
exon	13892	13963			
exon	14184	14219			
exon	14311	14355			
exon	14440	14472			
exon	14603	14749			
exon	15093	15147			
exon	15467	15655			
exon	16387	16464			
exon	16895	17606			
CDS	GB:AF019406_1651412CD1	2067 bp	32 exons	#147	
exon	1115	1189			
exon	2635	2709			
exon	3905	3940			
exon	4025	4087			
exon	5507	5560			



TABLE 1 (Cont.)

exon	5682	5717				
exon	5811	5834				
exon	6178	6231				
exon	6573	6626				
exon	6741	6788				
exon	7002	7058				
exon	7142	7195				
exon	7521	7574				
exon	7971	8024				
exon	8124	8177				
exon	8297	8350				
exon	10041	10094				
exon	10530	10583				
exon	10787	10840				
exon	12101	12145				
exon	12519	12572				
exon	13436	13489				
exon	13754	13807				
exon	13892	13963				
exon	14184	14219				
exon	14311	14355				
exon	14440	14472				
exon	14603	14749				
exon	15093	15147				
exon	15467	15655				
exon	16387	16464				
exon	16895	17091				
Allele	GB:AF019406	146	10809	10809	A>G	
	source	isSNP	SNP00032502			
	consequence	GB:AF019406_3482334CD1	148	Missense		330-330
Q>R						
	consequence	GB:AF019406_1651412CD1	147	Missense		326-326
Q>R						
Allele	GB:AF019406	146	13783	13783	A>G	
	source	isSNP	SNP00107343			
	consequence	GB:AF019406_3482334CD1	148	Silent		401-401
G						
	consequence	GB:AF019406_1651412CD1	147	Silent		397-397
G						
Allele	GB:AF019406	146	17229	17229	A>G	
	source	isSNP	SNP00032503			
	consequence	GB:AF019406_3482334CD1	148	3'		
	consequence	GB:AF019406_1651412CD1	147	3'		
GIF	COL9A2-genomic-fwd.gif					

## COMP

Full name : cartilage oligomeric matrix protein

Link : FL\_1901242\_link\_cdna

Subsequence FN:1901242CB1 1 2447 #151

CDS FN:1901242CB1.1 2274 bp #152

ORF 23 2296

Allele FN:1901242CB1 151 1200 1200 A&gt;G

source isSNP SNP00017026  
187

TABLE 1 (Cont.)

Allele	consequence	FN:1901242CB1.1	152	Missense	393-393	S>L
	FN:1901242CB1	151 1319	1319	C>G		
	source	isSNP SNP00108392				
Allele	consequence	FN:1901242CB1.1	152	Missense	433-433	D>H
	FN:1901242CB1	151 1335	1335	C>G		
	source	isSNP SNP00017027				
Allele	consequence	FN:1901242CB1.1	152	Missense	438-438	G>A
	FN:1901242CB1	151 1777	1777	A>G		
	source	isSNP SNP00017029				
	consequence	FN:1901242CB1.1	152	Silent	585-585	T
GIF COMP-cdna-fwd.gif						
Link : FL_1901242_link_genomic						
Subsequence	GB:AC003107	1	46275	#153		
Subsequence	GB:AC003107_1901242CD1		32077 23724	#154		
Subsequence	FL_1901242_mrna_build.1		32099 23582	#155		
CDS	GB:AC003107_1901242CD1	2274 bp	19 exons	#154		
exon	32077	31999				
exon	31743	31658				
exon	31421	31370				
exon	30922	30750				
exon	30105	29968				
exon	29721	29647				
exon	29558	29400				
exon	29322	29218				
exon	29127	29020				
exon	28458	28299				
exon	27459	27341				
exon	27100	27048				
exon	26955	26774				
exon	26660	26482				
exon	26355	26307				
exon	25901	25705				
exon	25172	25000				
exon	24002	23863				
exon	23770	23724				
mRNA	FL_1901242_mrna_build.1	2438 bp	19 exons	#155		
exon	32099	31999				
exon	31743	31658				
exon	31421	31370				
exon	30922	30750				
exon	30105	29968				
exon	29721	29647				
exon	29558	29400				
exon	29322	29218				
exon	29127	29020				
exon	28458	28299				
exon	27459	27341				
exon	27100	27048				
exon	26955	26774				
exon	26660	26482				
exon	26355	26307				
exon	25901	25705				
exon	25172	25000				
exon	24002	23863				
exon	23770	23582				

TABLE 1 (Cont.)

Allele	GB:AC003107	153	25864	25864	A>G			
	source	isSNP	SNP00017029					
	consequence	GB:AC003107_1901242CD1	154	Silent		585-585		
T								
Allele	GB:AC003107	153	27417	27417	A>G			
	source	isSNP	SNP00017026					
	consequence	GB:AC003107_1901242CD1	154	Missense		393-393		
S>L								
Allele	GB:AC003107	153	32082	32082	A>G			
	source	isSNP	SNP00017025					
	consequence	GB:AC003107_1901242CD1	154	5'				
GIF COMP-genomic-rev.gif								

## CRLF1

Full name : cytokine receptor-like factor 1

Link : CRLF1\_link\_cdna

Subsequence	GB:AF073515_1	1	1804	#156				
CDS	GB:AF073515_1.1	1269 bp		#157				
ORF	204	1472						
Allele	GB:AF073515_1	156	984	984	A>G			
	source	isSNP	SNP00015261					
	consequence	GB:AF073515_1.1	157	Missense		261-261	P>S	
GIF CRLF1-cdna-fwd.gif								

## CRP

Full name : C-reactive protein

Link : CRP\_link\_cdna

Subsequence	GB:X56214_1	1	1631	#158				
CDS	GB:X56214_1.1	675 bp		#159				
ORF	90	764						
Allele	GB:X56214_1	158	447	447	A>G			
	source	isSNP	SNP00100892					
	consequence	GB:X56214_1.1	159	Missense		120-120	S>P	
Allele	GB:X56214_1	158	988	988	A>G			
	source	isSNP	SNP00029575					
	consequence	GB:X56214_1.1	159	3'				
Allele	GB:X56214_1	158	1010	1010	A>G			
	source	isSNP	SNP00076237					
	consequence	GB:X56214_1.1	159	3'				
Allele	GB:X56214_1	158	1146	1146	C>G			
	source	isSNP	SNP00076238					
	consequence	GB:X56214_1.1	159	3'				
Allele	GB:X56214_1	158	1175	1175	G>T			
	source	isSNP	SNP00100893					
	consequence	GB:X56214_1.1	159	3'				
Allele	GB:X56214_1	158	1406	1406	A>G			
	source	isSNP	SNP00100894					
	consequence	GB:X56214_1.1	159	3'				
Allele	GB:X56214_1	158	1525	1525	A>G			
	source	isSNP	SNP00100895					
	consequence	GB:X56214_1.1	189	159	3'			

TABLE 1 (Cont.)

GIF CRP-cdna-fwd.gif  
Link : CRP\_link\_genomic

Subsequence	GB:HUMCRPGA	1	2480	#160	
Allele	GB:HUMCRPGA	160	865	865	A>G
	source	isSNP	SNP00100892		
Allele	GB:HUMCRPGA	160	1404	1404	A>G
	source	isSNP	SNP00029575		
Allele	GB:HUMCRPGA	160	1426	1426	A>G
	source	isSNP	SNP00076237		
Allele	GB:HUMCRPGA	160	1562	1562	C>G
	source	isSNP	SNP00076238		
Allele	GB:HUMCRPGA	160	1591	1591	G>T
	source	isSNP	SNP00100893		
Allele	GB:HUMCRPGA	160	1822	1822	A>G
	source	isSNP	SNP00100894		
Allele	GB:HUMCRPGA	160	1941	1941	A>G
	source	isSNP	SNP00100895		
Allele	GB:HUMCRPGA	160	2045	2045	A>G
	source	isSNP	SNP00100896		
Allele	GB:HUMCRPGA	160	2159	2159	A>G
	source	isSNP	SNP00100897		
Allele	GB:HUMCRPGA	160	2260	2260	A>G
	source	isSNP	SNP00006286		

CRTL1

Full name : cartilage linking protein 1

Link : CTRL1\_link\_cdna

Subsequence	GB:HSU43328	1	1759	#161	
CDS	GB:HSU43328.1	1065 bp		#162	
ORF	118	1182			
Allele	GB:HSU43328	161	801	801	C>G
	source	isSNP	SNP00020236		
	consequence	GB:HSU43328.1	162	Silent	228-228 G
Allele	GB:HSU43328	161	1454	1454	A>G
	source	isSNP	SNP00002295		
	consequence	GB:HSU43328.1	162	3'	

GIF CTRL1-cdna-fwd.gif

CTSC

Full name : cathepsin C

Link : CTSC\_link\_cdna

Subsequence	GB:NM_001814	1	1838	#163	
CDS	GB:NM_001814.1	1392 bp		#164	
ORF	34	1425			
Allele	GB:NM_001814	163	491	491	A>G
	source	isSNP	SNP00006579		
	consequence	GB:NM_001814.1	164	Missense	153-153 T>I
Allele	GB:NM_001814	163	1206	1206	G>T
	source	isSNP	SNP00006580		
	consequence	GB:NM_001814.1	164	Silent	391-391 T
Allele	GB:NM_001814	163	1224	1224	A>G

TABLE 1 (Cont.)

	source	isSNP	SNP00105444				
	consequence	GB:NM_001814.1	164	Silent	397-397	F	
GIF CTSC-cdna-fwd.gif							
Link : CTSC_link_genomic							
Subsequence	CTSC_cds.1	150285	106619	#165			
Subsequence	CTSC_cds.2	150285	106619	#166			
Subsequence	GB:AC011088_8	1	164991	#167			
Subsequence	CTSC_mrna_build.1	150318	106206	#168			
CDS	CTSC_cds.1	1392 bp	7 exons	#165			
exon	150285	150114					
exon	147695	147550					
exon	125167	125001					
exon	121931	121776					
exon	113258	113143					
exon	108877	108746					
exon	107121	106619					
CDS	CTSC_cds.2	1260 bp	6 exons	#166			
exon	150285	150114					
exon	147695	147550					
exon	125167	125001					
exon	121931	121776					
exon	113258	113143					
exon	107121	106619					
mRNA	CTSC_mrna_build.1	1838 bp	7 exons	#168			
exon	150318	150114					
exon	147695	147550					
exon	125167	125001					
exon	121931	121776					
exon	113258	113143					
exon	108877	108746					
exon	107121	106206					
Allele	GB:AC011088_8	167	106820	106820	A>G		
	source	isSNP	SNP00105444				
	consequence	CTSC_cds.1	165	Silent	397-397	F	
	consequence	CTSC_cds.2	166	Silent	353-353	F	
Allele	GB:AC011088_8	167	106838	106838	G>T		
	source	isSNP	SNP00006580				
	consequence	CTSC_cds.1	165	Silent	391-391	T	
	consequence	CTSC_cds.2	166	Silent	347-347	T	
Allele	GB:AC011088_8	167	122438	122438	A>G		
	source	dbSNP	gnl dbSNP ss1078568_allele				
	source	dbSNP	gnl dbSNP ss1088590_allele				
	source	dbSNP	gnl dbSNP ss382670_allele				
	source	dbSNP	gnl dbSNP ss403413_allele				
	consequence	CTSC_cds.1	165	Intron			
	consequence	CTSC_cds.2	166	Intron			
Allele	GB:AC011088_8	167	124932	124932	A>T		
	source	wetSNP	GB:AC011088_8.v124932.A>T				
	consequence	CTSC_cds.1	165	Intron			
	consequence	CTSC_cds.2	166	Intron			
Allele	GB:AC011088_8	167	125028	125028	A>G		
	source	isSNP	SNP00006579				
	source	wetSNP	GB:AC011088_8.v125028.A>G				
	consequence	CTSC_cds.1	165	Missense	153-153	I>T	
	consequence	CTSC_cds.2	166	Missense	153-153	I>T	
		191					

TABLE 1 (Cont.)

Allele	GB:AC011088_8	167	142996	142996	A>G
	source	dbSNP	gnl dbSNP ss1530135_allele		
	consequence	CTSC_cds.1	165	Intron	
	consequence	CTSC_cds.2	166	Intron	
Allele	GB:AC011088_8	167	150261	150261	A>G
	source	wetSNP	GB:AC011088_8.v150261.G>A		
	consequence	CTSC_cds.1	165	Missense	9-9 L>F
	consequence	CTSC_cds.2	166	Missense	9-9 L>F
Allele	GB:AC011088_8	167	150303	150303	A>G
	source	isSNP	SNP00067426		
	consequence	CTSC_cds.1	165	5'	
	consequence	CTSC_cds.2	166	5'	
GIF CTSC-genomic-rev.gif					

## CTSL

Full name : cathepsin L

Link : CTSL\_link\_genomic

Subsequence	CTSL_cds.1	35962	179319	#169
Subsequence	GB:AL160279_2	1	186528	#170
Subsequence	CTSL_mrna_build.1	34477	179604	#171
Subsequence	CTSL_cds.2	35962	179319	#172
mRNA	CTSL_mrna_build.1	1577 bp	8 exons	#171
exon	34477	34756		
exon	35952	36087		
exon	36385	36507		
exon	36608	36754		
exon	36943	37167		
exon	37931	38093		
exon	38739	38856		
exon	179220	179604		
CDS	CTSL_cds.1	1002 bp	7 exons	#169
exon	35962	36087		
exon	36385	36507		
exon	36608	36754		
exon	36943	37167		
exon	37931	38093		
exon	38739	38856		
exon	179220	179319		
CDS	CTSL_cds.2	777 bp	6 exons	#172
exon	35962	36087		
exon	36385	36507		
exon	36608	36754		
exon	37931	38093		
exon	38739	38856		
exon	179220	179319		
Allele	GB:AL160279_2	170	35919	35919 C>G
	source	wetSNP	GB:AL160279_2.v35919.C>G	
	consequence	CTSL_cds.1	169	5'
	consequence	CTSL_cds.2	172	5'
Allele	GB:AL160279_2	170	36118	36118 A>G
	source	wetSNP	GB:AL160279_2.v36118.C>T	
	consequence	CTSL_cds.1	169	Intron
	consequence	CTSL_cds.2	172	Intron
			192	

TABLE 1 (Cont.)

Allele	GB:AL160279_2	170	36191	36191	G>T	
	source	wetSNP	GB:AL160279_2.v36191.C>A			
	consequence	CTSL_cds.1	169	Intron		
	consequence	CTSL_cds.2	172	Intron		
Allele	GB:AL160279_2	170	44998	44998	A>G	
	source	isSNP	SNP00043782			
	consequence	CTSL_cds.1	169	Intron		
	consequence	CTSL_cds.2	172	Intron		
Allele	GB:AL160279_2	170	45748	45748	A>G	
	source	isSNP	SNP00007530			
	consequence	CTSL_cds.1	169	Intron		
	consequence	CTSL_cds.2	172	Intron		
Allele	GB:AL160279_2	170	45833	45833	C>G	
	source	isSNP	SNP00100366			
	consequence	CTSL_cds.1	169	Intron		
	consequence	CTSL_cds.2	172	Intron		
Allele	GB:AL160279_2	170	46188	46188	A>G	
	source	isSNP	SNP00100365			
	consequence	CTSL_cds.1	169	Intron		
	consequence	CTSL_cds.2	172	Intron		
Allele	GB:AL160279_2	170	46599	46599	C>G	
	source	isSNP	SNP00061067			
	consequence	CTSL_cds.1	169	Intron		
	consequence	CTSL_cds.2	172	Intron		
Allele	GB:AL160279_2	170	46662	46662	C>G	
	source	isSNP	SNP00100364			
	consequence	CTSL_cds.1	169	Intron		
	consequence	CTSL_cds.2	172	Intron		
Allele	GB:AL160279_2	170	65760	65760	A>G	
	source	isSNP	SNP00048929			
	consequence	CTSL_cds.1	169	Intron		
	consequence	CTSL_cds.2	172	Intron		
Allele	GB:AL160279_2	170	81133	81133	A>G	
	source	dbSNP	gnl dbSNP ss920176_allele			
	source	dbSNP	gnl dbSNP ss1066694_allele			
	source	dbSNP	gnl dbSNP ss402532_allele			
	consequence	CTSL_cds.1	169	Intron		
	consequence	CTSL_cds.2	172	Intron		
Allele	GB:AL160279_2	170	104937	104937	A>G	
	source	isSNP	SNP00055641			
	consequence	CTSL_cds.1	169	Intron		
	consequence	CTSL_cds.2	172	Intron		
Allele	GB:AL160279_2	170	115466	115466	A>G	
	source	isSNP	SNP00100363			
	consequence	CTSL_cds.1	169	Intron		
	consequence	CTSL_cds.2	172	Intron		
Allele	GB:AL160279_2	170	127655	127655	A>T	
	source	dbSNP	gnl dbSNP ss810769_allele			
	consequence	CTSL_cds.1	169	Intron		
	consequence	CTSL_cds.2	172	Intron		
Allele	GB:AL160279_2	170	149731	149731	A>G	
	source	dbSNP	gnl dbSNP ss1452230_allele			
	consequence	CTSL_cds.1	169	Intron		
	consequence	CTSL_cds.2	172	Intron		
GIF CTSL-genomic-fwd.gif						

TABLE 1 (Cont.)

DAF

Full name : decay accelerating factor for complement

Link : DAF\_link\_genomic

Subsequence	DAF_cds.1	131174	169024	#173	
Subsequence	DAF_cds.2	131174	169024	#174	
Subsequence	GB:AC031978_3	1	170170	#175	
Subsequence	DAF_mrna_build.1	131109	169897	#176	
CDS	DAF_cds.1	1146 bp	10 exons	#173	
	exon	131174	131273		
	exon	131790	131975		
	exon	133967	134158		
	exon	135030	135129		
	exon	136160	136245		
	exon	140516	140704		
	exon	146101	146226		
	exon	146737	146817		
	exon	148808	148828		
	exon	168960	169024		
CDS	DAF_cds.2	1125 bp	9 exons	#174	
	exon	131174	131273		
	exon	131790	131975		
	exon	133967	134158		
	exon	135030	135129		
	exon	136160	136245		
	exon	140516	140704		
	exon	146101	146226		
	exon	146737	146817		
	exon	168960	169024		
mRNA	DAF_mrna_build.1	2084 bp	10 exons	#176	
	exon	131109	131273		
	exon	131790	131975		
	exon	133967	134158		
	exon	135030	135129		
	exon	136160	136245		
	exon	140516	140704		
	exon	146101	146226		
	exon	146737	146817		
	exon	148808	148828		
	exon	168960	169897		
Allele	GB:AC031978_3	175	132041	132041	A>G
	source	wetSNP	GB:AC031978_3.v132041.C>T		
	consequence	DAF_cds.1	173	Intron	
	consequence	DAF_cds.2	174	Intron	
Allele	GB:AC031978_3	175	146352	146352	A>G
	source	isSNP	SNP00072272		
	consequence	DAF_cds.1	173	Intron	
	consequence	DAF_cds.2	174	Intron	
Allele	GB:AC031978_3	175	146611	146611	A>G
	source	isSNP	SNP00072273		
	consequence	DAF_cds.1	173	Intron	
	consequence	DAF_cds.2	174	Intron	
Allele	GB:AC031978_3	175	146659	146659	A>G
	source	isSNP	SNP00030860		



TABLE 1 (Cont.)

	consequence	DAF_cds.1	173	Intron	
	consequence	DAF_cds.2	174	Intron	
Allele	GB:AC031978_3	175	165604	165604	A>G
	source	isSNP	SNP00102533		
	consequence	DAF_cds.1	173	Intron	
	consequence	DAF_cds.2	174	Intron	
Allele	GB:AC031978_3	175	165743	165743	A>G
	source	isSNP	SNP00102534		
	consequence	DAF_cds.1	173	Intron	
	consequence	DAF_cds.2	174	Intron	

GIF DAF-genomic-fwd.gif

## E2F6

Full name : E2F transcription factor 6

Link : E2F6\_link\_cdna

Subsequence	GB:AF041381	1	2027	#177
Allele	GB:AF041381	177	1399	1399 A>G
source	isSNP	SNP00002319		

## EGF

Full name : EGF

Link : EGF\_link\_cdna

Subsequence	GB:HSEGFRER	1	4871	#178
CDS	GB:HSEGFRER.1	3624 bp		#179
ORF	437	4060		
Allele	GB:HSEGFRER	178	4453	4453 A>G
source	isSNP	SNP00043643		
consequence	GB:HSEGFRER.1	179	3'	

GIF EGF-cdna-fwd.gif

Link : EGF\_link\_genomic

Subsequence	GB:AC005509	1	143391	#180
Subsequence	GB:AC004050	270590	143492	#181
Subsequence	EGF_cds.1	64892	166730	#182
Subsequence	EGF_mrna_build.1	64456	167538	#183
CDS	EGF_cds.1	3624 bp	24 exons	#182
exon	64892	65018		
exon	92502	92701		
exon	94810	94991		
exon	95398	95625		
exon	96629	96831		
exon	110868	110993		
exon	112423	112545		
exon	113419	113541		
exon	114729	114854		
exon	115957	116093		
exon	120527	120675		
exon	126259	126363		
exon	127568	127791		
exon	131528	131695		
exon	132382	132531		
exon	134978	135097		

TABLE 1 (Cont.)

exon	139300	139416			
exon	143859	143984			
exon	148522	148644			
exon	150008	150155			
exon	154954	155121			
exon	159780	159897			
exon	163427	163505			
exon	166477	166730			
mRNA	EGF_mrna_build.1	4868 bp	24 exons	#183	
exon	64456 65018				
exon	92502 92701				
exon	94810 94991				
exon	95398 95625				
exon	96629 96831				
exon	110868	110993			
exon	112423	112545			
exon	113419	113541			
exon	114729	114854			
exon	115957	116093			
exon	120527	120675			
exon	126259	126363			
exon	127568	127791			
exon	131528	131695			
exon	132382	132531			
exon	134978	135097			
exon	139300	139416			
exon	140140	140265			
exon	148522	148644			
exon	150008	150155			
exon	154954	155121			
exon	159780	159897			
exon	163427	163505			
exon	166477	167538			
Allele	GB:AC005509	180 70903 70903 A>G			
	source	dbSNP gnl dbSNP ss875266_allele			
	consequence	EGF_cds.1 182 Intron			
Allele	GB:AC005509	180 92638 92638 A>G			
	source	wetSNP GB:AC005509.v92638.C>T			
	consequence	EGF_cds.1 182 Silent 88-88 I			
Allele	GB:AC005509	180 92670 92670 A>G			
	source	wetSNP GB:AC005509.v92670.A>G			
	consequence	EGF_cds.1 182 Missense 99-99 Q>R			
Allele	GB:AC005509	180 92763 92763 A>G			
	source	wetSNP GB:AC005509.v92763.C>T			
	consequence	EGF_cds.1 182 Intron			
Allele	GB:AC005509	180 94933 94933 A>G			
	source	wetSNP GB:AC005509.v94933.C>T			
	consequence	EGF_cds.1 182 Missense 151-151 H>Y			
Allele	GB:AC005509	180 95444 95444 C>G			
	source	wetSNP GB:AC005509.v95444.G>C			
	consequence	EGF_cds.1 182 Missense 186-186 D>H			
Allele	GB:AC005509	180 96578 96578 G>T			
	source	wetSNP GB:AC005509.v96578.A>C			
	consequence	EGF_cds.1 182 Intron			
Allele	GB:AC005509	180 96660 96660 C>G			

TABLE 1 (Cont.)

	source	wetSNP	GB:AC005509.v96660.G>C		
	consequence	EGF_cds.1	182 Missense	257-257	D>H
Allele	GB:AC005509	180 96842	96842 A>G		
	source	wetSNP	GB:AC005509.v96842.G>A		
	consequence	EGF_cds.1	182 Intron		
Allele	GB:AC005509	180 96853	96853 A>G		
	source	wetSNP	GB:AC005509.v96853.G>A		
	consequence	EGF_cds.1	182 Intron		
Allele	GB:AC005509	180 100795	100795 G>T		
	source	dbSNP gn1 dbSNP ss48546_allele			
	source	dbSNP gn1 dbSNP ss569965_allele			
	consequence	EGF_cds.1	182 Intron		
Allele	GB:AC005509	180 112451	112451 A>G		
	source	wetSNP	GB:AC005509.v112451.T>C		
	consequence	EGF_cds.1	182 Silent	365-365	H
Allele	GB:AC005509	180 113396	113396 A>G		
	source	wetSNP	GB:AC005509.v113396.T>C		
	consequence	EGF_cds.1	182 Intron		
Allele	GB:AC005509	180 113521	113521 A>G		
	source	wetSNP	GB:AC005509.v113521.G>A		
	consequence	EGF_cds.1	182 Missense	431-431	R>K
Allele	GB:AC005509	180 114696	114696 A>G		
	source	wetSNP	GB:AC005509.v114696.C>T		
	consequence	EGF_cds.1	182 Intron		
Allele	GB:AC005509	180 126323	126323 A>G		
	source	wetSNP	GB:AC005509.v126323.A>G		
	consequence	EGF_cds.1	182 Missense	597-597	I>V
Allele	GB:AC005509	180 127715	127715 A>G		
	source	wetSNP	GB:AC005509.v127715.C>T		
	consequence	EGF_cds.1	182 Silent	659-659	C
Allele	GB:AC005509	180 131547	131547 A>G		
	source	wetSNP	GB:AC005509.v131547.A>G		
	consequence	EGF_cds.1	182 Silent	691-691	A
Allele	GB:AC005509	180 131598	131598 A>G		
	source	wetSNP	GB:AC005509.v131598.G>A		
	consequence	EGF_cds.1	182 Missense	708-708	M>I
Allele	GB:AC005509	180 131641	131641 C>G		
	source	wetSNP	GB:AC005509.v131641.G>C		
	consequence	EGF_cds.1	182 Missense	723-723	G>R
Allele	GB:AC005509	180 132511	132511 A>T		
	source	wetSNP	GB:AC005509.v132511.A>T		
	consequence	EGF_cds.1	182 Missense	784-784	D>V
Allele	GB:AC005509	180 139281	139281 A>G		
	source	wetSNP	GB:AC005509.v139281.G>A		
	consequence	EGF_cds.1	182 Intron		
Allele	GB:AC005509	180 139333	139333 A>G		
	source	wetSNP	GB:AC005509.v139333.T>C		
	consequence	EGF_cds.1	182 Missense	842-842	M>T
Allele	GB:AC004050	181 126737	126737 G>T		
	source	wetSNP	GB:AC004050.v126737.C>A		
	consequence	EGF_cds.1	182 Intron		
Allele	GB:AC004050	181 122948	122948 A>G		
	source	isSNP SNP00118827			
	consequence	EGF_cds.1	182 Intron		
Allele	GB:AC004050	181 122045	122045 A>T		

TABLE 1 (Cont.)

	source	wetSNP	GB:AC004050.v122045.A>T			
	consequence	EGF_cds.1	182	Missense	920-920	E>V
Allele	GB:AC004050	181	110980	110980	G>T	
	source	isSNP	SNP00101773			
	consequence	EGF_cds.1	182	Intron		
Allele	GB:AC004050	181	110796	110796	A>G	
	source	wetSNP	GB:AC004050.v110796.A>G			
	consequence	EGF_cds.1	182	Silent	1063-1063	L
Allele	GB:AC004050	181	104082	104083	GC>GCC	
	source	wetSNP	GB:AC004050.v104082.GC>GCC			
	consequence	EGF_cds.1	182	Frameshift	1134-1135	
Allele	GB:AC004050	181	103468	103468	A>G	
	source	isSNP	SNP00043643			
	consequence	EGF_cds.1	182	3'		

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## FDFT1

Full name : farnesyl-diphosphate farnesyltransferase 1

Link : FDFT1\_link\_cdna

Subsequence	GB:FDFT1	1	1649	#184		
CDS	GB:FDFT1.1	1254 bp		#185		
ORF	45	1298				
Allele	GB:FDFT1	184	65	65	A>G	
	source	isSNP	SNP00072434			
	consequence	GB:FDFT1.1	185	Silent	7-7	L
Allele	GB:FDFT1	184	178	178	A>G	
	source	isSNP	SNP00065489			
	consequence	GB:FDFT1.1	185	Missense	45-45	K>R
Allele	GB:FDFT1	184	245	245	A>G	
	source	isSNP	SNP00018570			
	consequence	GB:FDFT1.1	185	Silent	67-67	N
Allele	GB:FDFT1	184	590	590	A>G	
	source	isSNP	SNP00123116			
	consequence	GB:FDFT1.1	185	Silent	182-182	G
Allele	GB:FDFT1	184	1016	1016	C>G	
	source	isSNP	SNP00003188			
	consequence	GB:FDFT1.1	185	Silent	324-324	L
Allele	GB:FDFT1	184	1220	1220	A>G	
	source	isSNP	SNP00123117			
	consequence	GB:FDFT1.1	185	Silent	392-392	L
Allele	GB:FDFT1	184	1532	1532	A>G	
	source	isSNP	SNP00003189			
	consequence	GB:FDFT1.1	185	3'		

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Link : FDFT1\_link\_genomic

Subsequence	FDFT1_cds.1	5681	37973	#186		
Subsequence	GB:AC025857_2_000033		1	19420	#187	
Subsequence	GB:AC025857_2_000021		19521	25487	#188	
Subsequence	GB:AC025857_2_000014		29099	25588	#189	
Subsequence	GB:AC025857_2_000029		29200	40859	#190	
Subsequence	FDFT1_mrna_build.1		5639	38324	#191	
mRNA	FDFT1_mrna_build.1	1647 bp		8 exons		#191
exon	5639	5779				

TABLE 1 (Cont.)

exon	11642	11739							
exon	12515	12698							
exon	24238	24366							
exon	26209	26400							
exon	29608	29784							
exon	30882	31034							
exon	37752	38324							
CDS	FDFT1_cds.1	1254 bp	8 exons	#186					
exon	5681	5779							
exon	11642	11739							
exon	12515	12698							
exon	24238	24366							
exon	26209	26400							
exon	29608	29784							
exon	30882	31034							
exon	37752	37973							
Allele	GB:AC025857_2_000033	187	5701	5701	A>G				
	source	isSNP SNP00072434							
	consequence	FDFT1_cds.1 186	Silent		7-7	L			
Allele	GB:AC025857_2_000033	187	6103	6103	C>G				
	source	isSNP SNP00072231							
	consequence	FDFT1_cds.1 186	Intron						
Allele	GB:AC025857_2_000033	187	11676	11676	A>G				
	source	isSNP SNP00065489							
	consequence	FDFT1_cds.1 186	Missense		45-45	K>R			
Allele	GB:AC025857_2_000014	189	2856	2856	A>G				
	source	isSNP SNP00123116							
	consequence	FDFT1_cds.1 186	Silent		182-182	G			
Allele	GB:AC025857_2_000029	190	1775	1775	C>G				
	source	isSNP SNP00003188							
	consequence	FDFT1_cds.1 186	Silent		324-324	L			
Allele	GB:AC025857_2_000029	190	5704	5704	A>G				
	source	isSNP SNP00096026							
	consequence	FDFT1_cds.1 186	Intron						
Allele	GB:AC025857_2_000029	190	8528	8528	A>G				
	source	isSNP SNP00105147							
	consequence	FDFT1_cds.1 186	Intron						
Allele	GB:AC025857_2_000029	190	8696	8696	A>G				
	source	isSNP SNP00123117							
	consequence	FDFT1_cds.1 186	Silent		392-392	L			
Allele	GB:AC025857_2_000029	190	9008	9008	A>G				
	source	isSNP SNP00003189							
	consequence	FDFT1_cds.1 186	3'						
Allele	GB:AC025857_2_000029	190	9148	9148	G>T				
	source	isSNP SNP00003190							
	consequence	FDFT1_cds.1 186	3'						
GIF	FDFT1-genomic-fwd.gif								

## FGF1

Full name : Fibroblast growth factor 1 (acidic)

Link : FGF1\_link\_cdna

Subsequence GB:X51943\_1 1 2259 #192

CDS GB:X51943\_1.1 468 bp #193

TABLE 1 (Cont.)

ORF	35	502						
Allele	GB:X51943_1	192	590	590	A>G			
	source	isSNP	SNP00075582					
	consequence	GB:X51943_1.1		193	3'			
Allele	GB:X51943_1	192	785	785	G>T			
	source	isSNP	SNP00075583					
	consequence	GB:X51943_1.1		193	3'			
Allele	GB:X51943_1	192	1855	1855	A>G			
	source	isSNP	SNP00069845					
	consequence	GB:X51943_1.1		193	3'			
Allele	GB:X51943_1	192	2007	2007	C>G			
	source	isSNP	SNP00075584					
	consequence	GB:X51943_1.1		193	3'			
GIF FGF1-cdna-fwd.gif								
Link : FL_2535357_link_genomic								
Subsequence	GB:AC005370	1	76416	#194				
Subsequence	GB:AC005370_3284782CD1		45026	63860	#195			
Subsequence	FL_3284782_mrna_build.1		44979	67355	#196			
mRNA	FL_3284782_mrna_build.1	920 bp		4 exons	#196			
exon	44979	45194						
exon	58348	58451						
exon	63669	64259						
exon	67347	67355						
CDS	GB:AC005370_3284782CD1	465 bp		3 exons	#195			
exon	45026	45194						
exon	58348	58451						
exon	63669	63860						
Allele	GB:AC005370	194	63951	63951	A>G			
	source	isSNP	SNP00075582					
	consequence	GB:AC005370_3284782CD1		195	3'			
Allele	GB:AC005370	194	64146	64146	G>T			
	source	isSNP	SNP00075583					
	consequence	GB:AC005370_3284782CD1		195	3'			
Allele	GB:AC005370	194	65119	65119	G>T			
	source	isSNP	SNP00012384					
	consequence	GB:AC005370_3284782CD1		195	3'			
Allele	GB:AC005370	194	65217	65217	A>G			
	source	isSNP	SNP00069845					
	consequence	GB:AC005370_3284782CD1		195	3'			
Allele	GB:AC005370	194	65369	65369	C>G			
	source	isSNP	SNP00075584					
	consequence	GB:AC005370_3284782CD1		195	3'			
Allele	GB:AC005370	194	66005	66005	A>G			
	source	isSNP	SNP00045433					
	consequence	GB:AC005370_3284782CD1		195	3'			
GIF FGF1-genomic-fwd.gif								

## FGF2

Full name : fibroblast growth factor 2 (basic)

Link : FGF2\_link\_cdna

Subsequence	GB:FGF2	1	6757	#197
CDS	GB:FGF2.1	633 bp		#198
ORF	302	934		

TABLE 1 (Cont.)

Allele	GB:FGF2	197	1651	1651	G>T
	source	isSNP	SNP00023270		
	consequence	GB:FGF2.1	198	3'	
Allele	GB:FGF2	197	1691	1691	A>G
	source	isSNP	SNP00058183		
	consequence	GB:FGF2.1	198	3'	
Allele	GB:FGF2	197	4603	4603	A>G
	source	isSNP	SNP00036340		
	consequence	GB:FGF2.1	198	3'	
Allele	GB:FGF2	197	4909	4909	A>G
	source	isSNP	SNP00036341		
	consequence	GB:FGF2.1	198	3'	
Allele	GB:FGF2	197	5455	5455	A>G
	source	isSNP	SNP00123025		
	consequence	GB:FGF2.1	198	3'	
Allele	GB:FGF2	197	5466	5466	C>G
	source	isSNP	SNP00036342		
	consequence	GB:FGF2.1	198	3'	
Allele	GB:FGF2	197	5892	5892	G>T
	source	isSNP	SNP00062439		
	consequence	GB:FGF2.1	198	3'	
Allele	GB:FGF2	197	5937	5937	A>G
	source	isSNP	SNP00062440		
	consequence	GB:FGF2.1	198	3'	

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## FGFR1

Full name : Fibroblast growth factor receptor-1

Link : FGFR1\_link\_cdna

Subsequence	GB:M34185_1	1	3365	#199	
CDS	GB:M34185_1.1	2202 bp		#200	
ORF	256	2457			
Allele	GB:M34185_1	199	1471	1471	A>G
	source	isSNP	SNP00107960		
	consequence	GB:M34185_1.1	200	Missense	406-406 A>T
Allele	GB:M34185_1	199	3224	3224	G>T
	source	isSNP	SNP00107961		
	consequence	GB:M34185_1.1	200	3'	

GIF FGFR1-cdna-fwd.gif

## FMOD

Full name : fibromodulin

Link : FMOD\_link\_cdna

Subsequence	GB:FMOD	1	2863	#201
CDS	GB:FMOD.1	1131 bp	#202	
ORF	21	1151		
Allele	GB:FMOD	201	2653	2653 C>G
	source	isSNP	SNP00001499	
	consequence	GB:FMOD.1	202	3'
Allele	GB:FMOD	201	2739	2739 A>G
	source	isSNP	SNP00001500	
		201		

TABLE 1 (Cont.)

consequence		GB:FMOD.1	202	3'			
GIF FMOD-cdna-fwd.gif							
FRZB							
Full name : frizzled-related protein							
Link : FRZB_link_cdna							
Subsequence	GB:U91903_1	1	1909	#203			
CDS	GB:U91903_1.1	978 bp		#204			
ORF	70	1047					
Allele	GB:U91903_1	203	667	667	A>G		
	source	isSNP	SNP00016790				
	consequence	GB:U91903_1.1	204	Missense	200-200	R>W	
Allele	GB:U91903_1	203	1039	1039	C>G		
	source	isSNP	SNP00001065				
	consequence	GB:U91903_1.1	204	Missense	324-324	R>G	
Allele	GB:U91903_1	203	1259	1259	A>G		
	source	isSNP	SNP00001066				
	consequence	GB:U91903_1.1	204	3'			
Allele	GB:U91903_1	203	1305	1305	A>G		
	source	isSNP	SNP00016791				
	consequence	GB:U91903_1.1	204	3'			
GIF FRZB-cdna-fwd.gif							
FST							
Full name : Follistatin							
Link : FST_link_cdna							
Subsequence	GB:FST	1	954	#205			
CDS	GB:FST.1	954 bp		#206			
ORF	1	954					
Allele	GB:FST	205	454	454	A>G		
	source	isSNP	SNP00015508				
	consequence	GB:FST.1	206	Missense	152-152	E>K	
Allele	GB:FST	205	853	853	C>G		
	source	isSNP	SNP00052278				
	consequence	GB:FST.1	206	Missense	285-285	A>P	
GIF FST-cdna-fwd.gif							
Link : FST_link_genomic							
Subsequence	FST_cds.1	77877	73442	#207			
Subsequence	GB:AC008901_2	1	192639	#208			
Subsequence	FST_mrna_build.1	77877	73440	#209			
CDS	FST_cds.1	951 bp	5 exons	#207			
exon	77877	77793					
exon	75788	75597					
exon	75164	74946					
exon	74599	74375					
exon	73671	73442					
mRNA	FST_mrna_build.1	953 bp	5 exons	#209			
exon	77877	77793					
exon	75788	75597					
exon	75164	74946					
exon	74599	74375					



TABLE 1 (Cont.)

exon	73671	73440					
Allele	GB:AC008901_2	208	73454	73454	A>G		
	source	wetSNP	GB:AC008901_2.v73454.G>A				
	consequence	FST_cds.1	207	Silent		313-313	S
Allele	GB:AC008901_2	208	73540	73540	C>G		
	source	isSNP	SNP00052278				
	consequence	FST_cds.1	207	Missense		285-285	A>P
Allele	GB:AC008901_2	208	74988	74988	A>G		
	source	isSNP	SNP00015508				
	consequence	FST_cds.1	207	Missense		152-152	E>K
Allele	GB:AC008901_2	208	76361	76361	C>G		
	source	dbSNP	gnl dbSNP ss42460_allele				
	consequence	FST_cds.1	207	Intron			
Allele	GB:AC008901_2	208	76373	76373	A>G		
	source	dbSNP	gnl dbSNP ss1048607_allele				
	source	dbSNP	gnl dbSNP ss226044_allele				
	consequence	FST_cds.1	207	Intron			
Allele	GB:AC008901_2	208	76384	76384	A>G		
	source	dbSNP	gnl dbSNP ss839844_allele				
	consequence	FST_cds.1	207	Intron			

GIF FST-genomic-rev.gif

## G0S2

Full name : putative lymphocyte G0\G1 switch gene

Link : FL\_3732868\_link\_genomic

Subsequence	GB:HS28010	1	97700	#210	
Subsequence	GB:HS28010_3732868CD1		52369	52680	#211
Subsequence	FL_3732868_mrna_build.1		52008	53073	#212
mRNA	FL_3732868_mrna_build.1	963 bp		2 exons	#212
exon	52008	52233			
exon	52337	53073			
CDS	GB:HS28010_3732868CD1	312 bp		1 exon	#211
exon	52369	52680			
Allele	GB:HS28010	210	52341	52341	A>G
	source	isSNP	SNP00039143		
	source	wetSNP	GB:HS28010.v52341.T>C		
	consequence	GB:HS28010_3732868CD1	211	5'	

GIF G0S2-genomic-fwd.gif

## GADD34

Full name : growth arrest and DNA-damage-inducible 34

Link : GADD34\_link\_cdna

Subsequence	GB:HSU83981	1	2331	#213	
CDS	GB:HSU83981.1	2025 bp		#214	
ORF	223	2247			
Allele	GB:HSU83981	213	205	205	A>G
	source	isSNP	SNP00116263		
	consequence	GB:HSU83981.1		214	5'
Allele	GB:HSU83981	213	314	314	A>G
	source	isSNP	SNP00116264		
	consequence	GB:HSU83981.1	214	Missense	31-31 R>H

TABLE 1 (Cont.)

Allele	GB:HSU83981	213	316	316	A>G			
	source	isSNP	SNP00029694					
	consequence	GB:HSU83981.1		214	Missense	32-32	A>T	
Allele	GB:HSU83981	213	974	974	C>G			
	source	isSNP	SNP00006368					
	consequence	GB:HSU83981.1		214	Missense	251-251		R>P
Allele	GB:HSU83981	213	1051	1051	A>G			
	source	isSNP	SNP00006369					
	consequence	GB:HSU83981.1		214	Missense	277-277		K>E
Allele	GB:HSU83981	213	1156	1156	A>G			
	source	isSNP	SNP00006370					
	consequence	GB:HSU83981.1		214	Missense	312-312		G>S
Allele	GB:HSU83981	213	1605	1605	A>G			
	source	isSNP	SNP00069978					
	consequence	GB:HSU83981.1		214	Silent	461-461		L
Allele	GB:HSU83981	213	1650	1650	G>T			
	source	isSNP	SNP00069979					
	consequence	GB:HSU83981.1		214	Missense	476-476		R>S
Allele	GB:HSU83981	213	2011	2011	A>G			
	source	isSNP	SNP00006372					
	consequence	GB:HSU83981.1		214	Missense	597-597		T>A
Allele	GB:HSU83981	213	2184	2184	A>G			
	source	isSNP	SNP00006373					
	consequence	GB:HSU83981.1		214	Silent	654-654		A
Allele	GB:HSU83981	213	2199	2199	C>G			
	source	isSNP	SNP00006374					
	consequence	GB:HSU83981.1		214	Silent	659-659		S
GIF GADD34-cdna-fwd.gif								
Link : GADD34_link_genomic								
Subsequence	GADD34_cds.1		221390	224129		#215		
Subsequence	GB:AC026803_2		1	247509		#216		
Subsequence	GADD34_mrna_build.1		220595	224213		#217		
mRNA	GADD34_mrna_build.1		2331 bp	3 exons		#217		
exon	220595		220807					
exon	221381		223054					
exon	223770		224213					
CDS	GADD34_cds.1		2025 bp	2 exons		#215		
exon	221390		223054					
exon	223770		224129					
Allele	GB:AC026803_2		216	221481		221481		A>G
	source	isSNP	SNP00116264					
	consequence	GADD34_cds.1		215	Missense	31-31	R>H	
Allele	GB:AC026803_2		216	221483		221483		A>G
	source	isSNP	SNP00029694					
	consequence	GADD34_cds.1		215	Missense	32-32	A>T	
Allele	GB:AC026803_2		216	221941		221941		A>G
	source	wetSNP	GB:AC026803_2.v221941.G>A					
	consequence	GADD34_cds.1		215	Silent	184-184		R
Allele	GB:AC026803_2		216	221985		221985		A>G
	source	wetSNP	GB:AC026803_2.v221985.T>C					
	consequence	GADD34_cds.1		215	Missense	199-199		V>A
Allele	GB:AC026803_2		216	222141		222141		C>G
	source	isSNP	SNP00006368					
	source	wetSNP	GB:AC026803_2.v222141.G>C					
	consequence	GADD34_cds.1		215	Missense	251-251		R>P

TABLE 1 (Cont.)

Allele	GB:AC026803_2	216	222218	222218	A>G	
	source	isSNP	SNP00006369			
	consequence	GADD34_cds.1	215	Missense	277-277	K>E
Allele	GB:AC026803_2	216	222323	222323	A>G	
	source	isSNP	SNP00006370			
	consequence	GADD34_cds.1	215	Missense	312-312	G>S
Allele	GB:AC026803_2	216	222772	222772	A>G	
	source	isSNP	SNP00069978			
	consequence	GADD34_cds.1	215	Silent	461-461	L
Allele	GB:AC026803_2	216	222817	222817	G>T	
	source	isSNP	SNP00069979			
	consequence	GADD34_cds.1	215	Missense	476-476	R>S
Allele	GB:AC026803_2	216	223893	223893	A>G	
	source	isSNP	SNP00006372			
	consequence	GADD34_cds.1	215	Missense	597-597	T>A
Allele	GB:AC026803_2	216	224066	224066	A>G	
	source	isSNP	SNP00006373			
	consequence	GADD34_cds.1	215	Silent	654-654	A
Allele	GB:AC026803_2	216	224081	224081	C>G	
	source	isSNP	SNP00006374			
	consequence	GADD34_cds.1	215	Silent	659-659	S
GIF	GADD34-genomic-fwd.gif					

## GLI

Full name : glioma-associated oncogene homolog

Link : GLI\_link\_cdna

Subsequence	GB:NM_005269_1	1	3600	#218		
CDS	GB:NM_005269_1.1	3321 bp		#219		
ORF	79	3399				
Allele	GB:NM_005269_1	218	2179	2179	A>G	
	source	isSNP	SNP00018615			
	consequence	GB:NM_005269_1.1	219	Missense	701-701	R>G
Allele	GB:NM_005269_1	218	2202	2202	A>G	
	source	isSNP	SNP00072776			
	consequence	GB:NM_005269_1.1	219	Silent	708-708	E
Allele	GB:NM_005269_1	218	2876	2876	A>G	
	source	isSNP	SNP00112595			
	consequence	GB:NM_005269_1.1	219	Missense	933-933	G>D
Allele	GB:NM_005269_1	218	3243	3243	C>G	
	source	isSNP	SNP00018616			
	consequence	GB:NM_005269_1.1	219	Missense	1055-1055	E>D
Allele	GB:NM_005269_1	218	3376	3376	C>G	
	source	isSNP	SNP00018617			
	consequence	GB:NM_005269_1.1	219	Missense	1100-1100	E>Q
GIF	GLI-cdna-fwd.gif					

## GLI3

Full name : GLI-Kruppel family member GLI3

Link : GLI3\_link\_cdna

Subsequence	GB:NM_000168_1	1	5046	#220	
CDS	GB:NM_000168_1.1	4791 bp		#221	

TABLE 1 (Cont.)

ORF	55	4845					
Allele	GB:NM_000168_1	220	4502	4502	A>G		
	source	isSNP	SNP00031650				
	consequence	GB:NM_000168_1.1	221	Missense	1483-1483	G>D	
Allele	GB:NM_000168_1	220	4663	4663	A>G		
	source	isSNP	SNP00073523				
	consequence	GB:NM_000168_1.1	221	Missense	1537-1537	R>C	
GIF	GLI3-cdna-fwd.gif						

## HAS1

Full name : hyaluronan synthase 1

Link : HAS1\_link\_cdna

Subsequence	GB:NM_001523	1	2088	#222	
CDS	GB:NM_001523.1	1737 bp		#223	
ORF	36	1772			
Allele	GB:NM_001523	222	75	75	A>G
	source	isSNP	SNP00096015		
	consequence	GB:NM_001523.1	223	Missense	14-14 R>C
Allele	GB:NM_001523	222	1889	1889	G>T
	source	isSNP	SNP00064738		
	consequence	GB:NM_001523.1	223	3'	

GIF HAS1-cdna-fwd.gif

Link : HAS1\_link\_genomic

Subsequence	HAS1_cds.1	153154	142648	#224	
Subsequence	GB:AC018755_2	1	231222	#225	
Subsequence	HAS1_mrna_build.1	153189	142333	#226	
CDS	HAS1_cds.1	1737 bp	5 exons	#224	
exon	153154	153146			
exon	149119	148427			
exon	146414	146189			
exon	145609	145477			
exon	143323	142648			
mRNA	HAS1_mrna_build.1	2087 bp	5 exons	#226	
exon	153189	153146			
exon	149119	148427			
exon	146414	146189			
exon	145609	145477			
exon	143323	142333			
Allele	GB:AC018755_2	225	142531	142531	G>T
	source	isSNP	SNP00064738		
	consequence	HAS1_cds.1	224	3'	
Allele	GB:AC018755_2	225	147775	147775	G>T
	source	dbSNP	gnl dbSNP ss715930_allele		
	consequence	HAS1_cds.1	224	Intron	
Allele	GB:AC018755_2	225	149089	149089	A>G
	source	isSNP	SNP00096015		
	consequence	HAS1_cds.1	224	Missense	14-14 C>R
Allele	GB:AC018755_2	225	149293	149293	C>G
	source	dbSNP	gnl dbSNP ss713606_allele		
	consequence	HAS1_cds.1	224	Intron	

GIF HAS1-genomic-rev.gif

TABLE 1 (Cont.)

## HAS2

Full name : hyaluronan synthase 2

Link : HAS2\_link\_cdna

Subsequence	GB:NM_005328	1	3003	#227		
CDS	GB:NM_005328.1	1659 bp		#228		
ORF	536	2194				
Allele	GB:NM_005328	227	381	381	A>G	
	source	isSNP	SNP00072998			
	consequence	GB:NM_005328.1	228	5'		
Allele	GB:NM_005328	227	1357	1357	G>T	
	source	isSNP	SNP00104961			
	consequence	GB:NM_005328.1	228	Missense	274-274	F>L

GIF HAS2-cdna-fwd.gif

## HSPG2

Full name : heparan sulfate proteoglycan 2

Link : HSPG2\_link\_cdna

Subsequence	GB:NM_005529_2	1	13793	#229		
CDS	GB:NM_005529_2.1	13182 bp		#230		
ORF	41	13222				
Allele	GB:NM_005529_2	229	2155	2155	A>G	
	source	isSNP	SNP00054627			
	consequence	GB:NM_005529_2.1	230	Silent	705-705	A
Allele	GB:NM_005529_2	229	2340	2340	A>G	
	source	isSNP	SNP00054628			
	consequence	GB:NM_005529_2.1	230	Missense	767-767	S>N
Allele	GB:NM_005529_2	229	3603	3603	A>G	
	source	isSNP	SNP00109135			
	consequence	GB:NM_005529_2.1	230	Missense	1188-1188	R>Q
Allele	GB:NM_005529_2	229	3734	3734	A>G	
	source	isSNP	SNP00109136			
	consequence	GB:NM_005529_2.1	230	Missense	1232-1232	G>S
Allele	GB:NM_005529_2	229	3943	3943	A>G	
	source	isSNP	SNP00054629			
	consequence	GB:NM_005529_2.1	230	Silent	1301-1301	V
Allele	GB:NM_005529_2	229	4032	4032	A>G	
	source	isSNP	SNP00054630			
	consequence	GB:NM_005529_2.1	230	Missense	1331-1331	G>D
Allele	GB:NM_005529_2	229	4554	4554	A>G	
	source	isSNP	SNP00109138			
	consequence	GB:NM_005529_2.1	230	Missense	1505-1505	V>A
Allele	GB:NM_005529_2	229	7042	7042	A>G	
	source	isSNP	SNP00048871			
	consequence	GB:NM_005529_2.1	230	Silent	2334-2334	N
Allele	GB:NM_005529_2	229	7503	7503	A>G	
	source	isSNP	SNP00109139			
	consequence	GB:NM_005529_2.1	230	Missense	2488-2488	S>L
Allele	GB:NM_005529_2	229	9548	9548	A>G	
	source	isSNP	SNP00109140			
	consequence	GB:NM_005529_2.1	230	Missense	3170-3170	T>A
Allele	GB:NM_005529_2	229	10294	10294	A>G	
	source	isSNP	SNP00109141			
	consequence	GB:NM_005529_2.1	230	Silent	3418-3418	S

TABLE 1 (Cont.)

Allele	GB:NM_005529_2	229	10663	10663	A>G		
	source	isSNP	SNP00109142				
	consequence	GB:NM_005529_2.1	230	Silent		3541-3541	V
Allele	GB:NM_005529_2	229	10941	10941	A>G		
	source	isSNP	SNP00109143				
	consequence	GB:NM_005529_2.1	230	Missense		3634-3634	Q>R
Allele	GB:NM_005529_2	229	11233	11233	G>T		
	source	isSNP	SNP00009830				
	consequence	GB:NM_005529_2.1	230	Silent		3731-3731	V
Allele	GB:NM_005529_2	229	12358	12358	A>G		
	source	isSNP	SNP00009831				
	consequence	GB:NM_005529_2.1	230	Silent		4106-4106	D
Allele	GB:NM_005529_2	229	12604	12604	A>G		
	source	isSNP	SNP00038416				
	consequence	GB:NM_005529_2.1	230	Silent		4188-4188	S
GIF HSPG2-cdna-fwd.gif							

## IBSP

Full name: IBSP

Link: IBSP\_link\_cdna

Subsequence	GB:HUMSIALO	1	1037	#231			
CDS	GB:HUMSIALO.1	954 bp		#232			
ORF	72	1025					
Allele	GB:HUMSIALO	231	494	494	A>G		
	source	isSNP	SNP00065793				
	consequence	GB:HUMSIALO.1	232	Silent		141-141	N
Allele	GB:HUMSIALO	231	655	655	A>G		
	source	isSNP	SNP00065794				
	consequence	GB:HUMSIALO.1	232	Missense		195-195	G>E
Allele	GB:HUMSIALO	231	709	709	A>G		
	source	isSNP	SNP00018906				
	consequence	GB:HUMSIALO.1	232	Missense		213-213	G>D

GIF IBSP-cdna-fwd.gif

Link: IBSP\_link\_genomic

Subsequence	GB:HUMBNSP01	1	2415	#233			
Subsequence	GB:HUMBNSP02	2516	3359	#234			
Subsequence	GB:HUMBNSP03	3460	5094	#235			
Subsequence	GB:HUMBNSP04	5195	9497	#236			
Subsequence	IBSP_cds.1	2863	7195	#237			
CDS	IBSP_cds.1	954 bp	6 exons	#237			
exon	2863	2916					
exon	3009	3059					
exon	3158	3235					
exon	3571	3633					
exon	5882	6040					
exon	6647	7195					
Allele	GB:HUMBNSP04	236	1631	1631	A>G		
	source	isSNP	SNP00065794				
	consequence	IBSP_cds.1	237	Missense		195-195	E>G
Allele	GB:HUMBNSP04	236	1685	1685	A>G		
	source	isSNP	SNP00018906				
	consequence	IBSP_cds.1	237	Missense		213-213	G>D

GIF IBSP-genomic-fwd.gif

TABLE 1 (Cont.)

## IER3

Full name : immediate early response 3

Link : IER3\_link\_cdna

Subsequence	GB:Y14551_1	1	1230	#238
CDS	GB:Y14551_1.1	471 bp		#239
ORF	12	482		
Allele	GB:Y14551_1	238	838	838 A>G
source	isSNP	SNP00052893		
consequence	GB:Y14551_1.1	239	3'	

GIF IER3-cdna-fwd.gif

Link : FL\_758754\_link\_genomic

Subsequence	GB:AC006165	1	44118	#240
Subsequence	GB:AC006165_2619577CD1	14601	15183	#241
Subsequence	FL_2619577_mrna_build.1	14585	15920	#242
mRNA	FL_2619577_mrna_build.1	1224 bp	2 exons	#242
exon	14585	14810		
exon	14923	15920		
CDS	GB:AC006165_2619577CD1	471 bp	2 exons	#241
exon	14601	14810		
exon	14923	15183		
Allele	GB:AC006165	240	15539	15539 A>G
source	isSNP	SNP00052893		
consequence	GB:AC006165_2619577CD1	241	3'	

GIF IER3-genomic-fwd.gif

## IHH

Full name : IHH

Link : IHH\_link\_cdna

Subsequence	GB:HUMIHH	1	1277	#243
CDS	GB:HUMIHH.2	939 bp		#244
ORF	2	940		
Allele	GB:HUMIHH	243	457	457 A>G
source	isSNP	SNP00097225		
consequence	GB:HUMIHH.2	244	Silent	152-152 P

GIF IHH-cdna-fwd.gif

Link : IHH\_link\_genomic

Subsequence	IHH_cds.1	1	1469	#245
Subsequence	GB:AB010092_1	1	315	#246
Subsequence	GB:AB018075_1	416	698	#247
Subsequence	GB:AB018076_1	799	1481	#248
CDS	IHH_cds.1	1236 bp	3 exons	#245
exon	1	315		
exon	426	687		
exon	811	1469		
Allele	GB:AB018075_1	247	194	194 A>G
source	wetSNP	GB:AB018075_1.v194	G>A	
consequence	IHH_cds.1	245	Missense	167-167 A>T
Allele	GB:AB018076_1	248	188	188 A>G
source	isSNP	SNP00097225		
consequence	IHH_cds.1	245	Silent	251-251 P

GIF IHH-genomic-fwd.gif

TABLE 1 (Cont.)

## INHBA

Full name : inhibin, beta A

Link : FL\_3526170\_link\_cdna

Subsequence	FN:3526170CB1	1	1620	#249	
CDS	FN:3526170CB1.1	1281 bp		#250	
ORF	216	1496			
Allele	FN:3526170CB1	249	607	607	G>T
	source	isSNP	SNP00068777		
	consequence	FN:3526170CB1.1	250	Missense	131-131 T>K

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Link : FL\_3526170\_link\_genomic

Subsequence	GB:AC005027	1	199878	#251	
Subsequence	GB:AC005027_3526170CD1	16865	54957	#252	
Subsequence	FL_3526170_mrna_build.1	14163	55081	#253	
mRNA	FL_3526170_mrna_build.1	1620 bp	3 exons	#253	
exon	14163	14234			
exon	16722	17252			
exon	54065	55081			
CDS	GB:AC005027_3526170CD1	1281 bp	2 exons	#252	
exon	16865	17252			
exon	54065	54957			
Allele	GB:AC005027	251	16377	16377	A>G
	source	dbSNP	gnl dbSNP ss577365_allele		
	source	dbSNP	gnl dbSNP ss588511_allele		
	consequence	GB:AC005027_3526170CD1	252	5'	

GIF INHBA-genomic-fwd.gif

## IRS1

Full name : Insulin receptor substrate 1

Link : IRS1\_link\_cdna

Subsequence	EM:S62539	1	5828	#254	
CDS	EM:S62539.1	3729 bp		#255	
ORF	1021	4749			
Allele	EM:S62539	254	3388	3388	A>G
	source	isSNP	SNP00067005		
	consequence	EM:S62539.1	255	Missense	790-790 R>C
Allele	EM:S62539	254	3887	3887	A>G
	source	isSNP	SNP00114530		
	consequence	EM:S62539.1	255	Missense	956-956 E>G
Allele	EM:S62539	254	5156	5156	G>T
	source	isSNP	SNP00067006		
	consequence	EM:S62539.1	255	3'	

GIF IRS1-cdna-fwd.gif

Link : IRS1\_link\_genomic

Subsequence	EM:S85963	100	6251	#256	
Subsequence	IRS1_cds.1	680	4411	#257	
Subsequence	IRS1_mrna_build.1	100	4432	#258	
CDS	IRS1_cds.1	3732 bp	1 exon	#257	
exon	680	4411			
mRNA	IRS1_mrna_build.1	4333 bp	1 exon	#258	
exon	100	4432			



TABLE 1 (Cont.)

Allele	EM:S85963	256	850	850	A>G	
	source	wetSNP		EM:S85963.v850.C>T		
	consequence	IRS1_cds.1	257	Silent	90-90	D
Allele	EM:S85963	256	1285	1285	A>G	
	source	wetSNP		EM:S85963.v1285.G>A		
	consequence	IRS1_cds.1	257	Silent	235-235	G
Allele	EM:S85963	256	1783	1783	A>G	
	source	wetSNP		EM:S85963.v1783.T>C		
	consequence	IRS1_cds.1	257	Silent	401-401	H
Allele	EM:S85963	256	2023	2023	A>G	
	source	wetSNP		EM:S85963.v2023.C>T		
	consequence	IRS1_cds.1	257	Silent	481-481	N
Allele	EM:S85963	256	2117	2117	C>G	
	source	wetSNP		EM:S85963.v2117.G>C		
	consequence	IRS1_cds.1	257	Missense	513-513	A>P
Allele	EM:S85963	256	2697	2697	A>G	
	source	wetSNP		EM:S85963.v2697.G>A		
	consequence	IRS1_cds.1	257	Missense	706-706	G>D
Allele	EM:S85963	256	2941	2941	A>G	
	source	wetSNP		EM:S85963.v2941.T>C		
	consequence	IRS1_cds.1	257	Silent	787-787	H
Allele	EM:S85963	256	2951	2951	A>G	
	source	isSNP SNP00067005				
	consequence	IRS1_cds.1	257	Missense	791-791	R>C
Allele	EM:S85963	256	2995	2995	A>G	
	source	wetSNP		EM:S85963.v2995.A>G		
	consequence	IRS1_cds.1	257	Silent	805-805	A
Allele	EM:S85963	256	3035	3035	C>G	
	source	wetSNP		EM:S85963.v3035.G>C		
	consequence	IRS1_cds.1	257	Missense	819-819	G>R
Allele	EM:S85963	256	3262	3262	C>G	
	source	wetSNP		EM:S85963.v3262.G>C		
	consequence	IRS1_cds.1	257	Silent	894-894	P
Allele	EM:S85963	256	3349	3349	A>G	
	source	wetSNP		EM:S85963.v3349.G>A		
	consequence	IRS1_cds.1	257	Silent	923-923	R
Allele	EM:S85963	256	3450	3450	A>G	
	source	isSNP SNP00114530				
	consequence	IRS1_cds.1	257	Missense	957-957	E>G
Allele	EM:S85963	256	3494	3494	A>G	
	source	wetSNP		EM:S85963.v3494.G>A		
	consequence	IRS1_cds.1	257	Missense	972-972	G>R
Allele	EM:S85963	256	4053	4053	A>G	
	source	wetSNP		EM:S85963.v4053.G>A		
	consequence	IRS1_cds.1	257	Missense	1158-1158	G>E
GIF IRS1-genomic-fwd.gif						

JUN

Full name : v-jun avian sarcoma virus 17 oncogene homolog

Link : JUN\_link\_genomic

Subsequence	JUN_cds.1	9468	8473	#259	
Subsequence	GB:AL136985_1		1	151212	#260
Subsequence	JUN_mrna_build.1	9468	8473	#261	

TABLE 1 (Cont.)

CDS JUN\_cds.1 996 bp 1 exon #259  
 exon 9468 8473  
 mRNA JUN\_mrna\_build.1 996 bp 1 exon #261  
 exon 9468 8473  
 GIF JUN-genomic-rev.gif

KJ\_OA11

Full name : KIAA1253

Link : FL\_2135776\_link\_cdna

Subsequence	FN:2135776CB1	1	3129	#262		
CDS	FN:2135776CB1.1	1197 bp		#263		
ORF	256 1452					
Allele	FN:2135776CB1	262 59	59	C>G		
	source isSNP	SNP00100733				
	consequence	FN:2135776CB1.1	263	5'		
Allele	FN:2135776CB1	262 1352	1352	A>G		
	source isSNP	SNP00116557				
	consequence	FN:2135776CB1.1	263	Missense	366-366	Q>R
Allele	FN:2135776CB1	262 1477	1477	A>G		
	source isSNP	SNP00042286				
	consequence	FN:2135776CB1.1	263	3'		
Allele	FN:2135776CB1	262 1489	1489	A>G		
	source isSNP	SNP00042287				
	consequence	FN:2135776CB1.1	263	3'		
Allele	FN:2135776CB1	262 1667	1667	A>G		
	source isSNP	SNP00011480				
	consequence	FN:2135776CB1.1	263	3'		
Allele	FN:2135776CB1	262 1710	1710	A>G		
	source isSNP	SNP00011481				
	consequence	FN:2135776CB1.1	263	3'		
Allele	FN:2135776CB1	262 1838	1838	A>G		
	source isSNP	SNP00011482				
	consequence	FN:2135776CB1.1	263	3'		
Allele	FN:2135776CB1	262 2589	2589	A>G		
	source isSNP	SNP00003671				
	consequence	FN:2135776CB1.1	263	3'		

GIF KJ\_OA11-cdna-fwd.gif

Link : FL\_2135776\_link\_genomic

Subsequence	GB:HS425C14	1	160203	#264	
Subsequence	GB:HS425C14_2135776CD1		55766 42255	#265	
Subsequence	FL_2135776_mrna_build.1		69012 40562	#266	
Subsequence	KJ_OA11_cds.1		55766 51052	#267	
CDS	GB:HS425C14_2135776CD1	1197 bp	9 exons	#265	
exon	55766 55731				
exon	53861 53692				
exon	51441 51362				
exon	51118 50981				
exon	49268 49099				
exon	48965 48875				
exon	44476 44332				
exon	44215 43985				
exon	42390 42255				
mRNA	FL_2135776_mrna_build.1	3119 bp	10 exons	#266	
		212			

TABLE 1 (Cont.)

exon	69012	68910						
exon	55892	55731						
exon	53861	53692						
exon	51441	51362						
exon	51118	50981						
exon	49268	49099						
exon	48965	48875						
exon	44476	44332						
exon	44215	43985						
exon	42390	40562						
CDS	KJ_OA11_cds.1	273 bp	3 exons	#267				
exon	55766	55731						
exon	53861	53692						
exon	51118	51052						
Allele	GB:HS425C14	264	41092	41092	A>G			
	source	isSNP	SNP00003671					
	consequence	GB:HS425C14_2135776CD1	265	3'				
	consequence	KJ_OA11_cds.1	267	3'				
Allele	GB:HS425C14	264	41843	41843	A>G			
	source	isSNP	SNP00011482					
	consequence	GB:HS425C14_2135776CD1	265	3'				
	consequence	KJ_OA11_cds.1	267	3'				
Allele	GB:HS425C14	264	41971	41971	A>G			
	source	isSNP	SNP00011481					
	consequence	GB:HS425C14_2135776CD1	265	3'				
	consequence	KJ_OA11_cds.1	267	3'				
Allele	GB:HS425C14	264	42014	42014	A>G			
	source	isSNP	SNP00011480					
	consequence	GB:HS425C14_2135776CD1	265	3'				
	consequence	KJ_OA11_cds.1	267	3'				
Allele	GB:HS425C14	264	42192	42192	A>G			
	source	isSNP	SNP00042287					
	consequence	GB:HS425C14_2135776CD1	265	3'				
	consequence	KJ_OA11_cds.1	267	3'				
Allele	GB:HS425C14	264	42204	42204	A>G			
	source	isSNP	SNP00042286					
	source	wetSNP	GB:HS425C14.v42204.G>A					
	consequence	GB:HS425C14_2135776CD1	265	3'				
	consequence	KJ_OA11_cds.1	267	3'				
Allele	GB:HS425C14	264	42294	42294	C>G			
	source	wetSNP	GB:HS425C14.v42294.G>C					
	source	wetSNP	GB:HS425C14.v42294.G>C					
	consequence	GB:HS425C14_2135776CD1	265	Silent			386-386	
L								
	consequence	KJ_OA11_cds.1	267	3'				
Allele	GB:HS425C14	264	42329	42329	A>G			
	source	isSNP	SNP00116557					
	consequence	GB:HS425C14_2135776CD1	265	Missense			375-375	
S>G								
	consequence	KJ_OA11_cds.1	267	3'				
Allele	GB:HS425C14	264	44297	44297	A>G			
	source	wetSNP	GB:HS425C14.v44297.T>C					
	consequence	GB:HS425C14_2135776CD1	265	Intron				
	consequence	KJ_OA11_cds.1	267	3'				
Allele	GB:HS425C14	264	55697	55697	A>G			

TABLE 1 (Cont.)

	source	wetSNP	GB:HS425C14.v55697.C>T		
	consequence	GB:HS425C14_2135776CD1	265	Intron	
	consequence	KJ_OA11_cds.1	267	Intron	
Allele	GB:HS425C14	264	68954	68954	C>G
	source	isSNP	SNP00100733		
	consequence	GB:HS425C14_2135776CD1	265	5'	
	consequence	KJ_OA11_cds.1	267	5'	

GIF KJ\_OA11-genomic-rev.gif

## KJ\_OA2

Link : KJ\_OA2\_link\_cdna

Subsequence	LG:244552.16	1	1825	#268
Allele	LG:244552.16	268	1476	1476 G>T
source	isSNP	SNP00098862		

## KJ\_OA21

Full name : FL project 2027624

Link : FL\_2027624\_link\_cdna

Subsequence	FN:2027624CB1	1	2173	#269	
CDS	FN:2027624CB1.1	1734 bp		#270	
ORF	4	1737			
Allele	FN:2027624CB1	269	881	881	C>G
	source	isSNP	SNP00106459		
	consequence	FN:2027624CB1.1	270	Missense	293-293 T>R
Allele	FN:2027624CB1	269	971	971	A>G
	source	isSNP	SNP00075286		
	consequence	FN:2027624CB1.1	270	Missense	323-323 T>I
Allele	FN:2027624CB1	269	1092	1092	C>G
	source	isSNP	SNP00106460		
	consequence	FN:2027624CB1.1	270	Silent	363-363 L
Allele	FN:2027624CB1	269	1254	1254	A>G
	source	isSNP	SNP00075287		
	consequence	FN:2027624CB1.1	270	Silent	417-417 Q
Allele	FN:2027624CB1	269	1374	1374	A>G
	source	isSNP	SNP00009699		
	consequence	FN:2027624CB1.1	270	Silent	457-457 T
Allele	FN:2027624CB1	269	1392	1392	A>G
	source	isSNP	SNP00097916		
	consequence	FN:2027624CB1.1	270	Silent	463-463 G
Allele	FN:2027624CB1	269	1623	1623	A>G
	source	isSNP	SNP00009700		
	consequence	FN:2027624CB1.1	270	Silent	540-540 Y

GIF KJ\_OA21-cdna-fwd.gif

Link : FL\_1250708\_link\_genomic

Subsequence	GB:HS453C12	1	147620	#271
Subsequence	GB:HS453C12_1394592CD1	87967	109084	#272
Subsequence	GB:HS453C12_2027624CD1	20194	10528	#273
Subsequence	FL_1394592_mrna_build.1	87945	110578	#274
Subsequence	FL_2027624_mrna_build.1	20197	6152	#275
Subsequence	OA21_cds.1	20194	17050	#276
mRNA	FL_2027624_mrna_build.1	2172 bp	13 exons	#275

TABLE 1 (Cont.)

exon	20197	20008							
exon	19834	19657							
exon	17499	17372							
exon	17056	16956							
exon	16847	16761							
exon	16215	16128							
exon	16019	15922							
exon	15823	15658							
exon	14968	14768							
exon	12135	11970							
exon	11855	11772							
exon	10777	10110							
exon	6168	6152							
CDS	OA21_cds.1	372 bp	3 exons	#276					
exon	20194	20008							
exon	19834	19657							
exon	17056	17050							
CDS	GB:HS453C12_2027624CD1	1734 bp	12 exons	#273					
exon	20194	20008							
exon	19834	19657							
exon	17499	17372							
exon	17056	16956							
exon	16847	16761							
exon	16215	16128							
exon	16019	15922							
exon	15823	15658							
exon	14968	14768							
exon	12135	11970							
exon	11855	11772							
exon	10777	10528							
Allele	GB:HS453C12	271	10642	10642	A>G				
source			isSNP	SNP00009700					
source			wetSNP	GB:HS453C12.v10642.A>G					
source			wetSNP	GB:HS453C12.v10642.A>G					
consequence	OA21_cds.1	276	3'						
consequence	GB:HS453C12_2027624CD1	273	Silent					540-540	
Y									
Allele	GB:HS453C12	271	11206	11206	A>G				
source			dbSNP	gnl dbSNP ss979258_allele					
consequence	OA21_cds.1	276	3'						
consequence	GB:HS453C12_2027624CD1	273	Intron						
Allele	GB:HS453C12	271	11999	11999	A>G				
source			isSNP	SNP00009699					
source			wetSNP	GB:HS453C12.v11999.C>T					
source			wetSNP	GB:HS453C12.v11999.C>T					
consequence	OA21_cds.1	276	3'						
consequence	GB:HS453C12_2027624CD1	273	Silent					457-457	
T									
Allele	GB:HS453C12	271	13494	13494	A>G				
source			isSNP	SNP00095042					
consequence	OA21_cds.1	276	3'						
consequence	GB:HS453C12_2027624CD1	273	Intron						
Allele	GB:HS453C12	271	14913	14913	C>G				
source			isSNP	SNP00106460					
consequence	OA21_cds.1	276	3'						
		215							

TABLE 1 (Cont.)

	consequence	GB:HS453C12_2027624CD1	273	Silent	363-363
L					
Allele	GB:HS453C12	271 15723 15723	A>G		
	source	isSNP SNP00075286			
	consequence	OA21_cds.1 276 3'			
	consequence	GB:HS453C12_2027624CD1	273	Missense	323-323
T>I					
GIF	KJ_OA21-genomic-rev.gif				

KJ\_OA29

Link : KJ\_OA29\_link\_cdna

Subsequence	LG:199489.1	1	3318	#277
Allele	LG:199489.1	277	544 544	A>G
	source	isSNP	SNP00005297	
Allele	LG:199489.1	277	695 695	A>G
	source	isSNP	SNP00121995	
Allele	LG:199489.1	277	971 971	A>G
	source	isSNP	SNP00047679	
Allele	LG:199489.1	277	1312 1312	A>G
	source	isSNP	SNP00005298	
Allele	LG:199489.1	277	1445 1445	A>G
	source	isSNP	SNP00027647	
Allele	LG:199489.1	277	2370 2370	A>G
	source	isSNP	SNP00005297	
Allele	LG:199489.1	277	2521 2521	A>G
	source	isSNP	SNP00121995	
Allele	LG:199489.1	277	2797 2797	A>G
	source	isSNP	SNP00047679	
Allele	LG:199489.1	277	3138 3138	A>G
	source	isSNP	SNP00005298	
Allele	LG:199489.1	277	3271 3271	A>G
	source	isSNP	SNP00027647	

KJ\_OA3

Link : KJ\_OA3\_link\_cdna

Subsequence	LG:153511.1	1	1628	#278
Allele	LG:153511.1	278	395 395	A>G
	source	isSNP	SNP00003503	
Allele	LG:153511.1	278	1101 1101	A>G
	source	isSNP	SNP00113687	

KJ\_OA31

Link : KJ\_OA31\_link\_cdna

Subsequence	LG:200972.2	1	2192	#279
Allele	LG:200972.2	279	366 366	C>G
	source	isSNP	SNP00099556	
Allele	LG:200972.2	279	836 836	A>G
	source	isSNP	SNP00015954	
Allele	LG:200972.2	279	1037 1037	A>G
			216	

TABLE 1 (Cont.)

	source	isSNP	SNP00015955	
Allele	LG:200972.2	279	1361 1361	A>G
	source	isSNP	SNP00000598	
Allele	LG:200972.2	279	1697 1697	A>G
	source	isSNP	SNP00000599	
Allele	LG:200972.2	279	1975 1975	A>G
	source	isSNP	SNP00067907	
Allele	LG:200972.2	279	2027 2027	A>G
	source	isSNP	SNP00067908	

## KJ\_OA33

Full name : cardiotrophin-like cytokine

Link : FL\_1676240\_link\_genomic

Subsequence	GB:AC005849_1	1	169144	#280	
Subsequence	KJ_OA33_cds.1	151862	143455	#281	
Subsequence	KJ_OA33_mrna_build.1	151907	142489	#282	
CDS	KJ_OA33_cds.1	678 bp	3 exons	#281	
exon	151862	151847			
exon	145945	145779			
exon	143949	143455			
mRNA	KJ_OA33_mrna_build.1	1689 bp	3 exons	#282	
exon	151907	151847			
exon	145945	145779			
exon	143949	142489			
GIF	KJ_OA33-genomic-rev.gif				

## KJ\_OA39

Link : KJ\_OA39\_link\_cdna

Subsequence	LG:293953.1	1	940	#283
Allele	LG:293953.1	283	679 679	G>T
	source	isSNP	SNP00110603	

## KJ\_OA6

Full name : FL project 2840746

Link : FL\_818498\_link\_genomic

Subsequence	GB:AC005598	1	190000	#284	
Subsequence	GB:AC005598_2840746CD1	132700	133368	#285	
Subsequence	FL_2840746_mrna_build.1	132672	135584	#286	
CDS	GB:AC005598_2840746CD1	669 bp	1 exon	#285	
exon	132700	133368			
mRNA	FL_2840746_mrna_build.1	1087 bp	2 exons	#286	
exon	132672	133391			
exon	135218	135584			
Allele	GB:AC005598	284	132689 132689	A>G	
	source	isSNP	SNP00005520		
	consequence	GB:AC005598_2840746CD1	285	5'	
Allele	GB:AC005598	284	132843 132843	A>G	
	source	wetSNP	GB:AC005598.v132843.C>T		
	consequence	GB:AC005598_2840746CD1	285	Silent	48-48 S

TABLE 1 (Cont.)

Allele	GB:AC005598	284	132878	132878	A>G	
	source	wetSNP	GB:AC005598.v132878.G>A			
	consequence	GB:AC005598_2840746CD1	285	Missense		60-60 R>H
Allele	GB:AC005598	284	132951	132951	A>G	
	source	wetSNP	GB:AC005598.v132951.C>T			
	consequence	GB:AC005598_2840746CD1	285	Silent		84-84 F
Allele	GB:AC005598	284	132967	132967	A>G	
	source	wetSNP	GB:AC005598.v132967.C>T			
	consequence	GB:AC005598_2840746CD1	285	Missense		90-90 P>S
Allele	GB:AC005598	284	133103	133103	G>T	
	source	wetSNP	GB:AC005598.v133103.G>T			
	consequence	GB:AC005598_2840746CD1	285	Missense		135-135
G>V						
Allele	GB:AC005598	284	133481	133481	A>G	
	source	wetSNP	GB:AC005598.v133481.C>T			
	consequence	GB:AC005598_2840746CD1	285	3'		
GIF KJ_OA6-genomic-fwd.gif						

KJ\_oagba3

Link : KJ\_oagba3\_link\_cdna

Subsequence	LG:215642.2	1	2849	#287
Allele	LG:215642.2	287	1475	1475 A>G
	source	isSNP	SNP00041601	
Allele	LG:215642.2	287	1963	1963 A>G
	source	isSNP	SNP00010951	

LIF

Full name : leukemia inhibitory factor

Link : LIF\_link\_cdna

Subsequence	GB:LIF	1	3848	#288
CDS	GB:LIF.1	609 bp		#289
ORF	45	653		
Allele	GB:LIF	288	1183	1183 G>T
	source	isSNP	SNP00036337	
	consequence	GB:LIF.1	289	3'
Allele	GB:LIF	288	1572	1572 A>G
	source	isSNP	SNP00099092	
	consequence	GB:LIF.1	289	3'
Allele	GB:LIF	288	1996	1996 C>G
	source	isSNP	SNP00099093	
	consequence	GB:LIF.1	289	3'
Allele	GB:LIF	288	2062	2062 G>T
	source	isSNP	SNP00099094	
	consequence	GB:LIF.1	289	3'
Allele	GB:LIF	288	2404	2404 A>G
	source	isSNP	SNP00099095	
	consequence	GB:LIF.1	289	3'
Allele	GB:LIF	288	3156	3156 A>G
	source	isSNP	SNP00036338	
	consequence	GB:LIF.1	289	3'
Allele	GB:LIF	288	3582	3582 A>G
			218	



TABLE 1 (Cont.)

source isSNP SNP00008778  
 consequence GB:LIF.1 289 3'  
 GIF LIF-cdna-fwd.gif  
 Link : OSM\_link\_genomic  
 Subsequence GB:AC004264 1 47188 #290  
 Subsequence LIF\_cds.1 11398 8354 #291  
 Subsequence LIF\_mrna\_build.1 11442 5156 #292  
 CDS LIF\_cds.1 609 bp 3 exons #291  
 exon 11398 11380  
 exon 9636 9458  
 exon 8764 8354  
 mRNA LIF\_mrna\_build.1 3851 bp 3 exons #292  
 exon 11442 11380  
 exon 9636 9458  
 exon 8764 5156  
 Allele GB:AC004264 290 5420 5420 A>G  
 source isSNP SNP00008778  
 consequence LIF\_cds.1 291 3'  
 Allele GB:AC004264 290 5846 5846 A>G  
 source isSNP SNP00036338  
 consequence LIF\_cds.1 291 3'  
 Allele GB:AC004264 290 6598 6598 A>G  
 source isSNP SNP00099095  
 consequence LIF\_cds.1 291 3'  
 Allele GB:AC004264 290 6940 6940 G>T  
 source isSNP SNP00099094  
 consequence LIF\_cds.1 291 3'  
 Allele GB:AC004264 290 7006 7006 C>G  
 source isSNP SNP00099093  
 consequence LIF\_cds.1 291 3'  
 Allele GB:AC004264 290 7435 7435 A>G  
 source isSNP SNP00099092  
 consequence LIF\_cds.1 291 3'  
 Allele GB:AC004264 290 7824 7824 G>T  
 source isSNP SNP00036337  
 consequence LIF\_cds.1 291 3'  
 GIF LIF-genomic-rev.gif

LUM

Full name : lumican

Link : FL\_2676170\_link\_genomic

Subsequence GB:AC007115\_1 1 180821 #293  
 Subsequence GB:AC007115\_1\_3128106CD1 87417 92234 #294  
 Subsequence FL\_3128106\_mrna\_build.1 84719 92839 #295  
 mRNA FL\_3128106\_mrna\_build.1 1926 bp 3 exons #295  
 exon 84719 84998  
 exon 87396 88278  
 exon 92077 92839  
 CDS GB:AC007115\_1\_3128106CD1 1020 bp 2 exons #294  
 exon 87417 88278  
 exon 92077 92234  
 Allele GB:AC007115\_1 293 89050 89050 A>G  
 source dbSNP gnl|dbSNP|ss852530\_allele

TABLE 1 (Cont.)

```

source      dbSNP gnl|dbSNP|ss897123_allele
consequence GB:AC007115_1_3128106CD1      294      Intron
Allele      GB:AC007115_1      293      89249 89249 A>G
source      dbSNP gnl|dbSNP|ss855039_allele
consequence GB:AC007115_1_3128106CD1      294      Intron
GIF LUM-genomic-fwd.gif

```

## METTL1

Full name : methyltransferase-like 1

Link : METTL1\_link\_cdna

```

Subsequence  GB:Y18643_1 1      1292      #296
CDS GB:Y18643_1.1      831 bp      #297
ORF 49      879
Allele      GB:Y18643_1 296      345      345      A>G
source      isSNP SNP00098761
consequence GB:Y18643_1.1      297      Silent      99-99 P
Allele      GB:Y18643_1 296      919      919      A>G
source      isSNP SNP00003825
consequence GB:Y18643_1.1      297      3'
GIF METTL1-cdna-fwd.gif

```

## MMP1

Full name : matrix metalloproteinase 1

Link : MMP1\_link\_cdna

```

Subsequence  EM:HSCOLL1 1      1970      #298
Allele      EM:HSCOLL1 298      383      383      A>G
source      isSNP SNP00009627
Allele      EM:HSCOLL1 298      714      714      A>G
source      isSNP SNP00037857
Allele      EM:HSCOLL1 298      745      745      A>G
source      isSNP SNP00037858
Allele      EM:HSCOLL1 298      1522     1522     A>G
source      isSNP SNP00009628
Allele      EM:HSCOLL1 298      1541     1541     A>G
source      isSNP SNP00009629
Allele      EM:HSCOLL1 298      1662     1662     A>G
source      isSNP SNP00009630
Allele      EM:HSCOLL1 298      1747     1747     A>G
source      isSNP SNP00009631

```

Link : MMP1\_link\_genomic

```

Subsequence  GB:HSU78045 1      81826     #299
Subsequence  MMP1_cds.1 11905 4225     #300
Subsequence  MMP1_mrna_build.1 11973 3733     #301
CDS MMP1_cds.1 1410 bp      10 exons     #300
exon 11905 11801
exon 11314 11070
exon 10976 10828
exon 10603 10478
exon 9421 9266
exon 9105 8988
exon 6551 6418

```

TABLE 1 (Cont.)

exon	5308	5146						
exon	4619	4516						
exon	4334	4225						
mRNA	MMP1_mrna_build.1	1970 bp	10 exons	#301				
exon	11973	11801						
exon	11314	11070						
exon	10976	10828						
exon	10603	10478						
exon	9421	9266						
exon	9105	8988						
exon	6551	6418						
exon	5308	5146						
exon	4619	4516						
exon	4334	3733						
Allele	GB:HSU78045	299	3956	3956	A>G			
	source	isSNP	SNP00009631					
	consequence	MMP1_cds.1	300	3'				
Allele	GB:HSU78045	299	4041	4041	A>G			
	source	isSNP	SNP00009630					
	consequence	MMP1_cds.1	300	3'				
Allele	GB:HSU78045	299	4162	4162	A>G			
	source	isSNP	SNP00009629					
	consequence	MMP1_cds.1	300	3'				
Allele	GB:HSU78045	299	4181	4181	A>G			
	source	isSNP	SNP00009628					
	consequence	MMP1_cds.1	300	3'				
Allele	GB:HSU78045	299	4517	4517	A>G			
	source	wetSNP	GB:HSU78045.v4517.A>G					
	consequence	MMP1_cds.1	300	Silent	433-433	D		
Allele	GB:HSU78045	299	4661	4664	CATG>CG			
	source	wetSNP	GB:HSU78045.v4661.CATG>CG					
	consequence	MMP1_cds.1	300	Intron				
Allele	GB:HSU78045	299	4677	4677	A>G			
	source	wetSNP	GB:HSU78045.v4677.G>A					
	consequence	MMP1_cds.1	300	Intron				
Allele	GB:HSU78045	299	5198	5198	A>G			
	source	wetSNP	GB:HSU78045.v5198.A>G					
	consequence	MMP1_cds.1	300	Missense	382-382	S>P		
Allele	GB:HSU78045	299	6586	6586	A>G			
	source	wetSNP	GB:HSU78045.v6586.T>C					
	consequence	MMP1_cds.1	300	Intron				
Allele	GB:HSU78045	299	9056	9056	A>G			
	source	wetSNP	GB:HSU78045.v9056.C>T					
	consequence	MMP1_cds.1	300	Silent	277-277	A		
Allele	GB:HSU78045	299	9120	9120	A>G			
	source	wetSNP	GB:HSU78045.v9120.A>G					
	consequence	MMP1_cds.1	300	Intron				
Allele	GB:HSU78045	299	9126	9126	A>G			
	source	wetSNP	GB:HSU78045.v9126.G>A					
	consequence	MMP1_cds.1	300	Intron				
Allele	GB:HSU78045	299	9205	9205	A>G			
	source	wetSNP	GB:HSU78045.v9205.T>C					
	consequence	MMP1_cds.1	300	Intron				
Allele	GB:HSU78045	299	9247	9247	A>G			
	source	wetSNP	GB:HSU78045.v9247.T>C					

TABLE 1 (Cont.)

	consequence	MMP1_cds.1	300	Intron		
Allele	GB:HSU78045	299	9365	9365	G>T	
	source	wetSNP		GB:HSU78045.v9365.G>T		
	consequence	MMP1_cds.1	300	Missense	228-228	H>N
Allele	GB:HSU78045	299	9370	9370	A>G	
	source	isSNP	SNP00037858			
	consequence	MMP1_cds.1	300	Missense	226-226	L>P
Allele	GB:HSU78045	299	11105	11105	A>G	
	source	isSNP	SNP00009627			
	source	wetSNP		GB:HSU78045.v11105.C>T		
	consequence	MMP1_cds.1	300	Silent	105-105	G
GIF MMP1-genomic-rev.gif						

## MMP13

Full name : MMP13

Link : MMP13\_link\_genomic

Subsequence	MMP13_cds.1	141623		159614		#302
Subsequence	GB:AP000789_1	1		201766		#303
CDS	MMP13_cds.1	957 bp	7 exons			#302
exon	141629		141779			
exon	141956		142081			
exon	144063		144224			
exon	146009		146126			
exon	147078		147211			
exon	157208		157367			
exon	159509		159614			
Allele	GB:AP000789_1	303	141614	141614	C>G	
	source	wetSNP		GB:AP000789_1.v141614.C>G		
	consequence	MMP13_cds.1	302	5'		
Allele	GB:AP000789_1	303	141875	141875	G>T	
	source	wetSNP		GB:AP000789_1.v141875.C>A		
	consequence	MMP13_cds.1	302	Intron		
Allele	GB:AP000789_1	303	147095	147095	A>G	
	source	wetSNP		GB:AP000789_1.v147095.A>G		
	consequence	MMP13_cds.1	302	Missense	192-192	H>R
Allele	GB:AP000789_1	303	157231	157231	C>G	
	source	wetSNP		GB:AP000789_1.v157231.G>C		
	consequence	MMP13_cds.1	302	Missense	239-239	G>R
Allele	GB:AP000789_1	303	157325	157325	A>G	
	source	wetSNP		GB:AP000789_1.v157325.A>G		
	consequence	MMP13_cds.1	302	Missense	270-270	D>G
Allele	GB:AP000789_1	303	159631	159631	A>G	
	source	wetSNP		GB:AP000789_1.v159631.C>T		
	consequence	MMP13_cds.1	302	3'		
Allele	GB:AP000789_1	303	159644	159644	C>G	
	source	wetSNP		GB:AP000789_1.v159644.G>C		
	consequence	MMP13_cds.1	302	3'		
GIF MMP13-genomic-fwd.gif						

## MMP14

Full name : MMP14

TABLE 1 (Cont.)

Link : MMP14\_link\_cdna

Subsequence	GB:HUMMTMMP.1	1	3403	#304			
CDS	GB:HUMMTMMP.1	1749 bp		#305			
ORF	112	1860					
Allele	GB:HUMMTMMP	304	133	133	A>G		
	source	isSNP	SNP00107954				
	consequence	GB:HUMMTMMP.1	305	Missense	8-8	S>P	
Allele	GB:HUMMTMMP	304	580	580	A>G		
	source	isSNP	SNP00107955				
	consequence	GB:HUMMTMMP.1	305	Silent	157-157	L	
Allele	GB:HUMMTMMP	304	888	888	C>G		
	source	isSNP	SNP00093383				
	consequence	GB:HUMMTMMP.1	305	Silent	259-259	P	
Allele	GB:HUMMTMMP	304	966	966	A>G		
	source	isSNP	SNP00055171				
	consequence	GB:HUMMTMMP.1	305	Silent	285-285	G	
Allele	GB:HUMMTMMP	304	1243	1243	A>G		
	source	isSNP	SNP00107956				
	consequence	GB:HUMMTMMP.1	305	Missense	378-378	K>E	
Allele	GB:HUMMTMMP	304	1264	1264	C>G		
	source	isSNP	SNP00107957				
	consequence	GB:HUMMTMMP.1	305	Missense	385-385	D>H	
Allele	GB:HUMMTMMP	304	1944	1944	A>G		
	source	isSNP	SNP00060446				
	consequence	GB:HUMMTMMP.1	305	3'			

GIF MMP14-cdna-fwd.gif

Link : MMP14\_link\_genomic

Subsequence	MMP14_cds.1	132034	141254	#306		
Subsequence	GB:AL133448_3	1	173805	#307		
Subsequence	MMP14_mrna_build.1		131922	142801	#308	
CDS	MMP14_cds.1	1749 bp	10 exons	#306		
exon	132034	132141				
exon	136706	136854				
exon	137128	137250				
exon	137625	137932				
exon	138472	138633				
exon	138925	139085				
exon	139586	139724				
exon	139845	139995				
exon	140466	140581				
exon	140923	141254				
mRNA	MMP14_mrna_build.1	3408 bp	10 exons	#308		
exon	131922	132141				
exon	136706	136854				
exon	137128	137250				
exon	137625	137932				
exon	138472	138633				
exon	138925	139085				
exon	139586	139724				
exon	139845	139995				
exon	140466	140581				
exon	140923	142801				
Allele	GB:AL133448_3	307	132055	132055	A>G	
	source	isSNP	SNP00107954			
	consequence	MMP14_cds.1	306	Missense	8-8	P>S
			223			

TABLE 1 (Cont.)

Allele	GB:AL133448_3	307	137049	137051	TTA>TA	
	source	wetSNP	GB:AL133448_3.v137049	TTA>TA		
	consequence	MMP14_cds.1	306	Intron		
Allele	GB:AL133448_3	307	137713	137713	A>G	
	source	isSNP	SNP00107955			
	consequence	MMP14_cds.1	306	Silent	157-157	L
Allele	GB:AL133448_3	307	138406	138406	A>G	
	source	wetSNP	GB:AL133448_3.v138406	G>A		
	consequence	MMP14_cds.1	306	Intron		
Allele	GB:AL133448_3	307	138560	138560	C>G	
	source	isSNP	SNP00093383			
	source	wetSNP	GB:AL133448_3.v138560	C>G		
	consequence	MMP14_cds.1	306	Silent	259-259	P
Allele	GB:AL133448_3	307	138653	138653	A>G	
	source	wetSNP	GB:AL133448_3.v138653	G>A		
	consequence	MMP14_cds.1	306	Intron		
Allele	GB:AL133448_3	307	139639	139639	A>G	
	source	wetSNP	GB:AL133448_3.v139639	G>A		
	consequence	MMP14_cds.1	306	Missense	355-355	M>I
Allele	GB:AL133448_3	307	139981	139981	A>G	
	source	wetSNP	GB:AL133448_3.v139981	C>T		
	consequence	MMP14_cds.1	306	Silent	429-429	F
Allele	GB:AL133448_3	307	139986	139986	A>G	
	source	wetSNP	GB:AL133448_3.v139986	G>A		
	consequence	MMP14_cds.1	306	Missense	431-431	R>H
Allele	GB:AL133448_3	307	141337	141337	A>G	
	source	isSNP	SNP00060446			
	consequence	MMP14_cds.1	306	3'		
GIF MMP14-genomic-fwd.gif						

## MMP2

Link : MMP2\_link\_cdna

Subsequence	GB:HSMMPM2	1	3530	#309		
CDS	GB:HSMMPM2.1	2010 bp		#310		
ORF	49	2058				
Allele	GB:HSMMPM2	309	681	681	A>G	
	source	isSNP	SNP00100004			
	consequence	GB:HSMMPM2.1	310	Silent	211-211	P
Allele	GB:HSMMPM2	309	1835	1835	A>G	
	source	isSNP	SNP00100005			
	consequence	GB:HSMMPM2.1	310	Missense	596-596	D>G
Allele	GB:HSMMPM2	309	1851	1851	G>T	
	source	isSNP	SNP00075435			
	consequence	GB:HSMMPM2.1	310	Missense	601-601	F>L
Allele	GB:HSMMPM2	309	2717	2717	A>G	
	source	isSNP	SNP00024650			
	consequence	GB:HSMMPM2.1	310	3'		
Allele	GB:HSMMPM2	309	2922	2922	C>G	
	source	isSNP	SNP00024651			
	consequence	GB:HSMMPM2.1	310	3'		

GIF MMP2-cdna-fwd.gif

Link : MMP2\_link\_genomic

Subsequence	MMP2_cds.1	175558	224	156463	#311
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TABLE 1 (Cont.)

Subsequence	GB:AC012182_3	1	190117	#312	
Subsequence	MMP2_mrna_build.1	175606	155007	#313	
CDS	MMP2_cds.1	2010 bp	10 exons	#311	
exon	175558	175397			
exon	164437	164289			
exon	163643	163515			
exon	162034	161727			
exon	161372	161211			
exon	160292	160039			
exon	159678	159540			
exon	158699	158549			
exon	158397	158282			
exon	156902	156463			
mRNA	MMP2_mrna_build.1	3514 bp	10 exons	#313	
exon	175606	175397			
exon	164437	164289			
exon	163643	163515			
exon	162034	161727			
exon	161372	161211			
exon	160292	160039			
exon	159678	159540			
exon	158699	158549			
exon	158397	158282			
exon	156902	155007			
Allele	GB:AC012182_3	312	155598	155598	C>G
	source	isSNP	SNP00024651		
	consequence	MMP2_cds.1	311	3'	
Allele	GB:AC012182_3	312	155804	155804	A>G
	source	isSNP	SNP00024650		
	consequence	MMP2_cds.1	311	3'	
Allele	GB:AC012182_3	312	156670	156670	G>T
	source	isSNP	SNP00075435		
	consequence	MMP2_cds.1	311	Missense	601-601 F>L
Allele	GB:AC012182_3	312	156686	156686	A>G
	source	isSNP	SNP00100005		
	consequence	MMP2_cds.1	311	Missense	596-596 D>G
Allele	GB:AC012182_3	312	161842	161842	A>G
	source	isSNP	SNP00100004		
	consequence	MMP2_cds.1	311	Silent	211-211 P
Allele	GB:AC012182_3	312	163660	163660	A>G
	source	wetSNP	GB:AC012182_3.v163660.G>A		
	consequence	MMP2_cds.1	311	Intron	
GIF MMP2-genomic-rev.gif					

## MMP3

Full name : matrix metalloproteinase 3

Link : MMP3\_link\_cdna

Subsequence	EM:HSSTROMR	1	1801	#314
Allele	EM:HSSTROMR	314	331	331 A>G
	source	isSNP	SNP00011525	
Allele	EM:HSSTROMR	314	382	382 A>G
	source	isSNP	SNP00113489	
Allele	EM:HSSTROMR	314	713	713 A>G
			225	

TABLE 1 (Cont.)

	source	isSNP	SNP00015044			
Allele	EM:HSSTROMR	314	976	976	A>G	
	source	isSNP	SNP00054705			
Allele	EM:HSSTROMR	314	1129	1129	A>G	
	source	isSNP	SNP00011527			
Link : MMP3_link_genomic						
Subsequence	EM:HSU78045	100	81925	#315		
Subsequence	MMP3_link_cds.1		57437	50020	#316	
Subsequence	MMP3_mrna_build.1		57480	49696	#317	
CDS	MMP3_link_cds.1	1434 bp	10 exons		#316	
exon	57437	57333				
exon	56806	56562				
exon	56469	56321				
exon	56182	56057				
exon	54487	54323				
exon	54146	54002				
exon	53137	53004				
exon	52604	52445				
exon	51295	51192				
exon	50120	50020				
mRNA	MMP3_mrna_build.1	1801 bp	10 exons		#317	
exon	57480	57333				
exon	56806	56562				
exon	56469	56321				
exon	56182	56057				
exon	54487	54323				
exon	54146	54002				
exon	53137	53004				
exon	52604	52445				
exon	51295	51192				
exon	50120	49696				
Allele	EM:HSU78045	315	52375	52375	A>G	
	source	wetSNP	EM:HSU78045.v52375.T>C			
	consequence	MMP3_link_cds.1	316	Silent	400-400	T
Allele	EM:HSU78045	315	52411	52411	A>G	
	source	wetSNP	EM:HSU78045.v52411.G>A			
	consequence	MMP3_link_cds.1	316	Silent	388-388	I
Allele	EM:HSU78045	315	52489	52489	A>G	
	source	wetSNP	EM:HSU78045.v52489.G>A			
	consequence	MMP3_link_cds.1	316	Silent	362-362	A
Allele	EM:HSU78045	315	52527	52530	GAGT>GT	
	source	wetSNP	EM:HSU78045.v52527.GAGT>GT			
	consequence	MMP3_link_cds.1	316	Intron		
Allele	EM:HSU78045	315	52586	52586	A>T	
	source	wetSNP	EM:HSU78045.v52586.T>A			
	consequence	MMP3_link_cds.1	316	Intron		
Allele	EM:HSU78045	315	53771	53771	A>T	
	source	wetSNP	EM:HSU78045.v53771.T>A			
	consequence	MMP3_link_cds.1	316	Intron		
Allele	EM:HSU78045	315	54077	54077	C>G	
	source	wetSNP	EM:HSU78045.v54077.C>G			
	consequence	MMP3_link_cds.1	316	Intron		
Allele	EM:HSU78045	315	54187	54187	A>G	
	source	wetSNP	EM:HSU78045.v54187.C>T			
	consequence	MMP3_link_cds.1	316	Intron		



TABLE 1 (Cont.)

Allele	EM:HSU78045	315	54402	54402	A>G		
	source	wetSNP	EM:HSU78045.v54402.C>T				
	consequence	MMP3_link_cds.1	316	Intron			
Allele	EM:HSU78045	315	56119	56119	A>G		
	source	wetSNP	EM:HSU78045.v56119.C>T				
	consequence	MMP3_link_cds.1	316	Intron			
Allele	EM:HSU78045	315	56507	56507	C>G		
	source	wetSNP	EM:HSU78045.v56507.G>C				
	consequence	MMP3_link_cds.1	316	Silent		102-102	T
Allele	EM:HSU78045	315	56525	56525	A>G		
	source	isSNP	SNP00011525				
	source	wetSNP	EM:HSU78045.v56525.G>A				
	consequence	MMP3_link_cds.1	316	Silent		96-96	D
Allele	EM:HSU78045	315	56680	56680	A>G		
	source	wetSNP	EM:HSU78045.v56680.C>T				
	consequence	MMP3_link_cds.1	316	Missense		45-45	E>K

GIF MMP3-genomic-rev.gif

## MMP9

Full name : matrix metalloproteinase 9

Link : MMP9\_link\_cdna

Subsequence	FN:522678CB1	1	2348	#318			
CDS	FN:522678CB1.1	2124 bp		#319			
ORF	33	2156					
Allele	FN:522678CB1	318	308	308	A>G		
	source	isSNP	SNP00101082				
	consequence	FN:522678CB1.1	319	Silent		92-92	K
Allele	FN:522678CB1	318	413	413	A>G		
	source	isSNP	SNP00101083				
	consequence	FN:522678CB1.1	319	Silent		127-127	N
Allele	FN:522678CB1	318	534	534	A>G		
	source	isSNP	SNP00101084				
	consequence	FN:522678CB1.1	319	Missense		168-168	I>V
Allele	FN:522678CB1	318	591	591	A>G		
	source	isSNP	SNP00101085				
	consequence	FN:522678CB1.1	319	Missense		187-187	L>F
Allele	FN:522678CB1	318	719	719	A>G		
	source	isSNP	SNP00101086				
	consequence	FN:522678CB1.1	319	Silent		229-229	A
Allele	FN:522678CB1	318	748	748	A>G		
	source	isSNP	SNP00021346				
	consequence	FN:522678CB1.1	319	Missense		239-239	R>H
Allele	FN:522678CB1	318	868	868	A>G		
	source	isSNP	SNP00002987				
	consequence	FN:522678CB1.1	319	Missense		279-279	Q>R
Allele	FN:522678CB1	318	1604	1604	A>G		
	source	isSNP	SNP00021347				
	consequence	FN:522678CB1.1	319	Silent		524-524	I
Allele	FN:522678CB1	318	1853	1853	G>T		
	source	isSNP	SNP00002988				
	consequence	FN:522678CB1.1	319	Silent		607-607	G
Allele	FN:522678CB1	318	2159	2159	A>G		
	source	isSNP	SNP00062663				

TABLE 1 (Cont.)

	consequence	FN:522678CB1.1	319	3'	
Allele	FN:522678CB1	318 2302	2302	A>G	
	source	isSNP SNP00021348			
	consequence	FN:522678CB1.1	319	3'	
GIF MMP9-cdna-fwd.gif					
Link : MMP9_link_genomic					
Subsequence	GB:HUMIVCOL01	1	764	#320	
Subsequence	GB:HUMIVCOL02	865	1117	#321	
Subsequence	GB:HUMIVCOL03	1218	1386	#322	
Subsequence	GB:HUMIVCOL04	1487	1635	#323	
Subsequence	GB:HUMIVCOL05	1736	1929	#324	
Subsequence	GB:HUMIVCOL06	2030	2223	#325	
Subsequence	GB:HUMIVCOL07	2324	2520	#326	
Subsequence	GB:HUMIVCOL08	2621	2796	#327	
Subsequence	GB:HUMIVCOL09	2897	3196	#328	
Subsequence	GB:HUMIVCOL10	3297	3456	#329	
Subsequence	GB:HUMIVCOL11	3557	3727	#330	
Subsequence	GB:HUMIVCOL12	3828	3951	#331	
Subsequence	GB:HUMIVCOL13	4052	4371	#332	
Subsequence	MMP9_cds.1	619 4180		#333	
Subsequence	MMP9_mrna_build.1	587	4371	#334	
CDS	MMP9_cds.1	2124 bp	13 exons	#333	
	exon	619 756			
	exon	875 1107			
	exon	1228 1376			
	exon	1497 1625			
	exon	1746 1919			
	exon	2040 2213			
	exon	2334 2510			
	exon	2631 2786			
	exon	2907 3186			
	exon	3307 3446			
	exon	3567 3717			
	exon	3838 3941			
	exon	4062 4180			
mRNA	MMP9_mrna_build.1	2348 bp	13 exons	#334	
	exon	587 756			
	exon	875 1107			
	exon	1228 1376			
	exon	1497 1625			
	exon	1746 1919			
	exon	2040 2213			
	exon	2334 2510			
	exon	2631 2786			
	exon	2907 3186			
	exon	3307 3446			
	exon	3567 3717			
	exon	3838 3941			
	exon	4061 4371			
Allele	GB:HUMIVCOL01	320 677	677	A>G	
	source	wetSNP	GB:HUMIVCOL01.v677.C>T		
	consequence	MMP9_cds.1	333	Missense	20-20 A>V
Allele	GB:HUMIVCOL02	321 148	148	A>G	
	source	isSNP SNP00101082			
	consequence	MMP9_cds.1	333 228	Silent	92-92 K

TABLE 1 (Cont.)

Allele	GB:HUMIVCOL04	323	49	49	A>G		
	source	isSNP	SNP00101085				
	consequence	MMP9_cds.1	333	Missense	187-187	L>F	
Allele	GB:HUMIVCOL05	324	48	48	A>G		
	source	isSNP	SNP00101086				
	consequence	MMP9_cds.1	333	Silent	229-229	A	
Allele	GB:HUMIVCOL05	324	77	77	A>G		
	source	isSNP	SNP00021346				
	consequence	MMP9_cds.1	333	Missense	239-239	R>H	
Allele	GB:HUMIVCOL09	328	252	252	A>G		
	source	isSNP	SNP00021347				
	consequence	MMP9_cds.1	333	Silent	524-524	I	
Allele	GB:HUMIVCOL11	330	81	81	G>T		
	source	isSNP	SNP00002988				
	consequence	MMP9_cds.1	333	Silent	607-607	G	
Allele	GB:HUMIVCOL13	332	87	87	A>G		
	source	wetSNP	GB:HUMIVCOL13.v87.G>A				
	consequence	MMP9_cds.1	333	Silent	694-694	V	
Allele	GB:HUMIVCOL13	332	132	132	A>G		
	source	wetSNP	GB:HUMIVCOL13.v132.C>T				
	consequence	MMP9_cds.1	333	3'			
Allele	GB:HUMIVCOL13	332	274	274	A>G		
	source	isSNP	SNP00021348				
	consequence	MMP9_cds.1	333	3'			

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## MSF

Full name : megakaryocyte stimulating factor

Link : MSF\_link\_cdna

Subsequence	GB:NM_005807	1	5041	#335		
CDS	GB:NM_005807.1	4215 bp		#336		
ORF	34	4248				
Allele	GB:NM_005807	335	1011	1011	A>G	
	source	isSNP	SNP00064566			
	consequence	GB:NM_005807.1	336	Silent	326-326	K
Allele	GB:NM_005807	335	2650	2650	A>G	
	source	isSNP	SNP00108532			
	consequence	GB:NM_005807.1	336	Missense	873-873	P>S
Allele	GB:NM_005807	335	3171	3171	A>G	
	source	isSNP	SNP00009620			
	consequence	GB:NM_005807.1	336	Silent	1046-1046	P
Allele	GB:NM_005807	335	4187	4187	A>G	
	source	isSNP	SNP00061665			
	consequence	GB:NM_005807.1	336	Missense	1385-1385	A>V
Allele	GB:NM_005807	335	4760	4760	A>G	
	source	isSNP	SNP00009621			
	consequence	GB:NM_005807.1	336	3'		

GIF MSF-cdna-fwd.gif

Link : MSF\_link\_genomic

Subsequence	MSF_cds.1	181003	197905	#337
Subsequence	MSF_cds.2	181003	197905	#338
Subsequence	MSF_cds.3	181003	197905	#339
Subsequence	MSF_cds.4	181003	197905	#340

TABLE 1 (Cont.)

Subsequence	GB:AL133553_7	1	214019	#341
Subsequence	MSF_mrna_build.1	180982	198681	#342
CDS MSF_cds.3	3936 bp	10 exons	#339	
exon	181003	181078		
exon	184218	184340		
exon	185719	185838		
exon	190445	193267		
exon	193920	193997		
exon	195161	195297		
exon	195567	195723		
exon	196302	196499		
exon	196896	197021		
exon	197808	197905		
mRNA	MSF_mrna_build.1	5012 bp	12 exons	#342
exon	180982	181078		
exon	184218	184340		
exon	185719	185838		
exon	188235	188384		
exon	188921	189049		
exon	190445	193267		
exon	193920	193997		
exon	195161	195297		
exon	195567	195723		
exon	196302	196499		
exon	196896	197021		
exon	197808	198681		
CDS MSF_cds.4	3813 bp	9 exons	#340	
exon	181003	181078		
exon	185719	185838		
exon	190445	193267		
exon	193920	193997		
exon	195161	195297		
exon	195567	195723		
exon	196302	196499		
exon	196896	197021		
exon	197808	197905		
CDS MSF_cds.1	4215 bp	12 exons	#337	
exon	181003	181078		
exon	184218	184340		
exon	185719	185838		
exon	188235	188384		
exon	188921	189049		
exon	190445	193267		
exon	193920	193997		
exon	195161	195297		
exon	195567	195723		
exon	196302	196499		
exon	196896	197021		
exon	197808	197905		
CDS MSF_cds.2	4092 bp	11 exons	#338	
exon	181003	181078		
exon	185719	185838		
exon	188235	188384		
exon	188921	189049		
exon	190445	193267		

TABLE 1 (Cont.)

exon	193920	193997				
exon	195161	195297				
exon	195567	195723				
exon	196302	196499				
exon	196896	197021				
exon	197808	197905				
Allele	GB:AL133553_7	341	190505	190505	G>T	
	source	wetSNP	GB:AL133553_7.v190505	A>C		
	consequence	MSF_cds.3	339	Missense	127-127	D>A
	consequence	MSF_cds.4	340	Missense	86-86	D>A
	consequence	MSF_cds.1	337	Missense	220-220	D>A
	consequence	MSF_cds.2	338	Missense	179-179	D>A
Allele	GB:AL133553_7	341	190559	190559	A>G	
	source	wetSNP	GB:AL133553_7.v190559	C>T		
	consequence	MSF_cds.3	339	Missense	145-145	T>M
	consequence	MSF_cds.4	340	Missense	104-104	T>M
	consequence	MSF_cds.1	337	Missense	238-238	T>M
	consequence	MSF_cds.2	338	Missense	197-197	T>M
Allele	GB:AL133553_7	341	190755	190755	A>G	
	source	wetSNP	GB:AL133553_7.v190755	G>A		
	consequence	MSF_cds.3	339	Silent	210-210	K
	consequence	MSF_cds.4	340	Silent	169-169	K
	consequence	MSF_cds.1	337	Silent	303-303	K
	consequence	MSF_cds.2	338	Silent	262-262	K
Allele	GB:AL133553_7	341	190824	190824	A>G	
	source	isSNP	SNP00064566			
	consequence	MSF_cds.3	339	Silent	233-233	K
	consequence	MSF_cds.4	340	Silent	192-192	K
	consequence	MSF_cds.1	337	Silent	326-326	K
	consequence	MSF_cds.2	338	Silent	285-285	K
Allele	GB:AL133553_7	341	192463	192463	A>G	
	source	isSNP	SNP00108532			
	consequence	MSF_cds.3	339	Missense	780-780	P>S
	consequence	MSF_cds.4	340	Missense	739-739	P>S
	consequence	MSF_cds.1	337	Missense	873-873	P>S
	consequence	MSF_cds.2	338	Missense	832-832	P>S
Allele	GB:AL133553_7	341	192984	192984	A>G	
	source	isSNP	SNP00009620			
	consequence	MSF_cds.3	339	Silent	953-953	P
	consequence	MSF_cds.4	340	Silent	912-912	P
	consequence	MSF_cds.1	337	Silent	1046-1046	P
	consequence	MSF_cds.2	338	Silent	1005-1005	P
Allele	GB:AL133553_7	341	193235	193235	A>G	
	source	wetSNP	GB:AL133553_7.v193235	A>G		
	consequence	MSF_cds.3	339	Missense	1037-1037	N>S
	consequence	MSF_cds.4	340	Missense	996-996	N>S
	consequence	MSF_cds.1	337	Missense	1130-1130	N>S
	consequence	MSF_cds.2	338	Missense	1089-1089	N>S
Allele	GB:AL133553_7	341	193258	193258	A>G	
	source	wetSNP	GB:AL133553_7.v193258	A>G		
	consequence	MSF_cds.3	339	Missense	1045-1045	M>V
	consequence	MSF_cds.4	340	Missense	1004-1004	M>V
	consequence	MSF_cds.1	337	Missense	1138-1138	M>V
	consequence	MSF_cds.2	338	Missense	1097-1097	M>V
Allele	GB:AL133553_7	341	196691	196691	G>T	

TABLE 1 (Cont.)

	source	isSNP	SNP00023429				
	consequence	MSF_cds.3	339	Intron			
	consequence	MSF_cds.4	340	Intron			
	consequence	MSF_cds.1	337	Intron			
	consequence	MSF_cds.2	338	Intron			
Allele	GB:AL133553_7	341	197844	197844		A>G	
	source	isSNP	SNP00061665				
	consequence	MSF_cds.3	339	Missense	1292-1292	A>V	
	consequence	MSF_cds.4	340	Missense	1251-1251	A>V	
	consequence	MSF_cds.1	337	Missense	1385-1385	A>V	
	consequence	MSF_cds.2	338	Missense	1344-1344	A>V	
Allele	GB:AL133553_7	341	198417	198417		A>G	
	source	isSNP	SNP00009621				
	consequence	MSF_cds.3	339	3'			
	consequence	MSF_cds.4	340	3'			
	consequence	MSF_cds.1	337	3'			
	consequence	MSF_cds.2	338	3'			
GIF MSF-genomic-fwd.gif							
NCOR2							
Full name : nuclear receptor co-repressor 2							
Link : NCOR2_link_cdna							
	Subsequence	GB:AF125672	1	8686	#343		
	CDS	GB:AF125672.1	7524 bp		#344		
	ORF	157	7680				
Allele	GB:AF125672	343	165	165	G>T		
	source	isSNP	SNP00035702				
	consequence	GB:AF125672.1	344	Silent	3-3	G	
Allele	GB:AF125672	343	618	618	A>G		
	source	isSNP	SNP00105557				
	consequence	GB:AF125672.1	344	Silent	154-154	P	
Allele	GB:AF125672	343	2859	2859	A>G		
	source	isSNP	SNP00101011				
	consequence	GB:AF125672.1	344	Silent	901-901	A	
Allele	GB:AF125672	343	4728	4728	A>G		
	source	isSNP	SNP00075034				
	consequence	GB:AF125672.1	344	Silent	1524-1524	G	
Allele	GB:AF125672	343	4749	4749	A>G		
	source	isSNP	SNP00069757				
	consequence	GB:AF125672.1	344	Silent	1531-1531	L	
Allele	GB:AF125672	343	4957	4957	A>G		
	source	isSNP	SNP00101012				
	consequence	GB:AF125672.1	344	Missense	1601-1601	Y>H	
Allele	GB:AF125672	343	5085	5085	A>G		
	source	isSNP	SNP00075035				
	consequence	GB:AF125672.1	344	Silent	1643-1643	R	
Allele	GB:AF125672	343	5100	5100	A>G		
	source	isSNP	SNP00075036				
	consequence	GB:AF125672.1	344	Silent	1648-1648	N	
Allele	GB:AF125672	343	5221	5221	A>G		
	source	isSNP	SNP00012485				
	consequence	GB:AF125672.1	344	Missense	1689-1689	T>A	
Allele	GB:AF125672	343	7405	7405	A>G		
			232				

TABLE 1 (Cont.)

	source	isSNP	SNP00015859				
	consequence	GB:AF125672.1	344	Missense	2417-2417	P>S	
Allele	GB:AF125672	343	7431 7431	A>G			
	source	isSNP	SNP00101013				
	consequence	GB:AF125672.1	344	Silent	2425-2425	S	
Allele	GB:AF125672	343	7751 7751	A>G			
	source	isSNP	SNP00101014				
	consequence	GB:AF125672.1	344	3'			
Allele	GB:AF125672	343	8597 8597	A>G			
	source	isSNP	SNP00062569				
	consequence	GB:AF125672.1	344	3'			
Allele	GB:AF125672	343	8602 8602	A>G			
	source	isSNP	SNP00012487				
	consequence	GB:AF125672.1	344	3'			

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NOG

Full name : NOG

Link : NOG\_link\_genomic

Subsequence	GB:AC005553	1	179651	#345		
Subsequence	NOG_cds.1	146202	145504	#346		
Subsequence	NOG_mrna_build.1	147012	145466	#347		
CDS	NOG_cds.1	699 bp	1 exon	#346		
exon	146202	145504				
mRNA	NOG_mrna_build.1	1547 bp	1 exon	#347		
exon	147012	145466				
Allele	GB:AC005553	345	145585	145585	A>G	
	source	wetSNP	GB:AC005553.v145585.G>A			
	consequence	NOG_cds.1	346	Silent	206-206	R

GIF NOG-genomic-rev.gif

NOTCH3

Link : NOTCH3\_link\_cdna

Subsequence	GB:NOTCH3	1	8091	#348		
CDS	GB:NOTCH3.1	6966 bp	#349			
ORF	79	7044				
Allele	GB:NOTCH3	348	1218 1218	A>G		
	source	isSNP	SNP00116668			
	consequence	GB:NOTCH3.1	349	Silent	380-380	P
Allele	GB:NOTCH3	348	1565 1565	A>G		
	source	isSNP	SNP00116669			
	consequence	GB:NOTCH3.1	349	Missense	496-496	P>L
Allele	GB:NOTCH3	348	2616 2616	A>G		
	source	isSNP	SNP00116670			
	consequence	GB:NOTCH3.1	349	Silent	846-846	C
Allele	GB:NOTCH3	348	4520 4520	A>G		
	source	isSNP	SNP00116671			
	consequence	GB:NOTCH3.1	349	Missense	1481-1481	D>G
Allele	GB:NOTCH3	348	5740 5740	A>G		
	source	isSNP	SNP00054178			
	consequence	GB:NOTCH3.1	349	Missense	1888-1888	F>L
			233			

TABLE 1 (Cont.)

Allele	GB:NOTCH3	348	6355	6355	A>G		
	source		isSNP	SNP00037780			
	consequence	GB:NOTCH3.1	349		Missense	2093-2093	A>T
Allele	GB:NOTCH3	348	6516	6516	A>G		
	source		isSNP	SNP00054179			
	consequence	GB:NOTCH3.1	349		Silent	2146-2146	A
Allele	GB:NOTCH3	348	6746	6746	A>G		
	source		isSNP	SNP00048081			
	consequence	GB:NOTCH3.1	349		Missense	2223-2223	V>A
Allele	GB:NOTCH3	348	7733	7733	A>G		
	source		isSNP	SNP00037781			
	consequence	GB:NOTCH3.1	349		3'		
Allele	GB:NOTCH3	348	7881	7881	A>G		
	source		isSNP	SNP00062225			
	consequence	GB:NOTCH3.1	349		3'		
Allele	GB:NOTCH3	348	7914	7914	A>G		
	source		isSNP	SNP00066446			
	consequence	GB:NOTCH3.1	349		3'		
Allele	GB:NOTCH3	348	8023	8023	A>G		
	source		isSNP	SNP00066447			
	consequence	GB:NOTCH3.1	349		3'		
GIF NOTCH3-cdna-fwd.gif							
Link : NOTCH3_link_genomic							
Subsequence	NOTCH3_cds.1		40735	3819	#350		
Subsequence	GB:AC004663_1		1	41150	#351		
CDS	NOTCH3_cds.1	6846 bp	32	exons	#350		
exon	40733	40657					
exon	35676	35534					
exon	35455	35117					
exon	35024	34902					
exon	34814	34581					
exon	32585	32430					
exon	32331	32146					
exon	31505	31392					
exon	31151	31038					
exon	30495	30262					
exon	30145	30035					
exon	28836	28644					
exon	28565	28414					
exon	28176	28063					
exon	27607	27452					
exon	24958	24733					
exon	24319	24118					
exon	23985	23838					
exon	23413	23229					
exon	22653	22521					
exon	22439	22182					
exon	22098	21980					
exon	21247	20682					
exon	17557	17225					
exon	13982	13828					
exon	13710	13488					
exon	13327	13243					
exon	10568	10406					
exon	9248	8944					



TABLE 1 (Cont.)

exon	8672	8525							
exon	5719	5622							
exon	4871	3819							
Allele	GB:AC004663_1	351	3796	3796	A>T				
	source	wetSNP	GB:AC004663_1.v3796.A>T						
	consequence	NOTCH3_cds.1	350	3'					
Allele	GB:AC004663_1	351	4117	4117	A>G				
	source	isSNP	SNP00048081						
	consequence	NOTCH3_cds.1	350	Missense	2183-2183	A>V			
Allele	GB:AC004663_1	351	4347	4347	A>G				
	source	isSNP	SNP00054179						
	consequence	NOTCH3_cds.1	350	Silent	2106-2106	A			
Allele	GB:AC004663_1	351	4508	4508	A>G				
	source	isSNP	SNP00037780						
	consequence	NOTCH3_cds.1	350	Missense	2053-2053	A>T			
Allele	GB:AC004663_1	351	5727	5727	A>G				
	source	wetSNP	GB:AC004663_1.v5727.A>G						
	consequence	NOTCH3_cds.1	350	Intron					
Allele	GB:AC004663_1	351	5943	5943	A>G				
	source	dbSNP	gnl dbSNP ss730238_allele						
	consequence	NOTCH3_cds.1	350	Intron					
Allele	GB:AC004663_1	351	17519	17519	A>G				
	source	isSNP	SNP00116671						
	consequence	NOTCH3_cds.1	350	Missense	1441-1441	D>G			
Allele	GB:AC004663_1	351	18749	18749	A>G				
	source	dbSNP	gnl dbSNP ss680542_allele						
	source	dbSNP	gnl dbSNP ss1143619_allele						
	source	dbSNP	gnl dbSNP ss372819_allele						
	consequence	NOTCH3_cds.1	350	Intron					
Allele	GB:AC004663_1	351	22353	22353	A>G				
	source	wetSNP	GB:AC004663_1.v22353.C>T						
	consequence	NOTCH3_cds.1	350	Missense	1143-1143	V>M			
Allele	GB:AC004663_1	351	23922	23922	C>G				
	source	wetSNP	GB:AC004663_1.v23922.C>G						
	consequence	NOTCH3_cds.1	350	Missense	980-980	A>P			
Allele	GB:AC004663_1	351	24045	24045	A>G				
	source	wetSNP	GB:AC004663_1.v24045.T>C						
	consequence	NOTCH3_cds.1	350	Intron					
Allele	GB:AC004663_1	351	27480	27480	A>G				
	source	isSNP	SNP00116670						
	consequence	NOTCH3_cds.1	350	Silent	806-806	C			
Allele	GB:AC004663_1	351	28173	28173	A>G				
	source	wetSNP	GB:AC004663_1.v28173.C>T						
	consequence	NOTCH3_cds.1	350	Missense	727-727	R>H			
Allele	GB:AC004663_1	351	28749	28749	A>G				
	source	wetSNP	GB:AC004663_1.v28749.C>T						
	consequence	NOTCH3_cds.1	350	Missense	640-640	R>H			
Allele	GB:AC004663_1	351	29997	29997	C>G				
	source	wetSNP	GB:AC004663_1.v29997.G>C						
	consequence	NOTCH3_cds.1	350	Intron					
Allele	GB:AC004663_1	351	32482	32482	A>G				
	source	isSNP	SNP00116668						
	consequence	NOTCH3_cds.1	350	Silent	340-340	P			
GIF NOTCH3-genomic-rev.gif									

TABLE 1 (Cont.)

## NPR2

Full name : Atrionatriuretic Peptide Receptor Type B

Link : NPR2\_link\_cdna

Subsequence	GB:HUMGUANCYC	1	4081	#352	
CDS	GB:HUMGUANCYC.2	3144 bp		#353	
ORF	651	3794			
Allele	GB:HUMGUANCYC	352	2222	2222	A>G
	source	isSNP	SNP00028343		
	consequence	GB:HUMGUANCYC.2	353	Silent	524-524 Y

GIF NPR2-cdna-fwd.gif

## OGN

Full name : osteoglycin

Link : OGN\_link\_cdna

Subsequence	GB:HSM801395	1	2101	#354	
CDS	GB:HSM801395.1	441 bp		#355	
ORF	1	441			
Allele	GB:HSM801395	354	64	64	A>G
	source	isSNP	SNP00100803		
	consequence	GB:HSM801395.1	355	Missense	22-22 L>F
Allele	GB:HSM801395	354	909	909	A>G
	source	isSNP	SNP00011097		
	consequence	GB:HSM801395.1	355	3'	

GIF OGN-cdna-fwd.gif

Link : OGN\_link\_genomic

Subsequence	OGN_cds.2	48897	32003	#356	
Subsequence	GB:AL354924_2	1	192427		#357
Subsequence	OGN_mrna_build.2	50083	30350	#358	
mRNA	OGN_mrna_build.2	2726 bp	7 exons		#358
exon	50083	49983			
exon	48969	48721			
exon	46672	46579			
exon	38619	38461			
exon	35431	35229			
exon	32679	32584			
exon	32173	30350			
CDS	OGN_cds.2	900 bp	6 exons	#356	
exon	48897	48721			
exon	46672	46579			
exon	38619	38461			
exon	35431	35229			
exon	32679	32584			
exon	32173	32003			
Allele	GB:AL354924_2	357	31535	31535	A>G
	source	isSNP	SNP00011097		
	consequence	OGN_cds.2	356	3'	
Allele	GB:AL354924_2	357	35339	35339	A>G
	source	isSNP	SNP00100803		
	consequence	OGN_cds.2	356	Missense	175-175 L>F

GIF OGN-genomic-rev.gif

TABLE 1 (Cont.)

OMD

Full name : osteomodulin

Link : OMD\_link\_cdna

Subsequence	GB:OMD	1	2263	#359		
CDS	GB:OMD.1	1266 bp		#360		
ORF	101	1366				
Allele	GB:OMD	359	159	159	C>G	
	source	isSNP	SNP00023658			
	consequence	GB:OMD.1	360	Missense	20-20	C>S
Allele	GB:OMD	359	762	762	A>G	
	source	isSNP	SNP00023659			
	consequence	GB:OMD.1	360	Missense	221-221	S>N
Allele	GB:OMD	359	1969	1969	A>G	
	source	isSNP	SNP00023660			
	consequence	GB:OMD.1	360	3'		
Allele	GB:OMD	359	2071	2071	G>T	
	source	isSNP	SNP00106046			
	consequence	GB:OMD.1	360	3'		

GIF OMD-cdna-fwd.gif

Link : FL\_1258977\_link\_genomic

Subsequence	GB:AB009589	1	12414	#361		
Subsequence	GB:AB009589_1258977CD1		8540	10946	#362	
Subsequence	FL_1258977_mrna_build.1	1685	11855	#363		
mRNA	FL_1258977_mrna_build.1	2396 bp		3 exons	#363	
exon	1685	1892				
exon	8524	9479				
exon	10624	11855				
CDS	GB:AB009589_1258977CD1	1263 bp		2 exons	#362	
exon	8540	9479				
exon	10624	10946				
Allele	GB:AB009589	361	8598	8598	C>G	
	source	isSNP	SNP00023658			
	consequence	GB:AB009589_1258977CD1	362	Missense	20-20	C>S
Allele	GB:AB009589	361	9201	9201	A>G	
	source	isSNP	SNP00023659			
	consequence	GB:AB009589_1258977CD1	362	Missense	221-221	
	S>N					
Allele	GB:AB009589	361	10042	10042	A>G	
	source	dbSNP	gnl dbSNP ss312223_allele			
	consequence	GB:AB009589_1258977CD1	362	Intron		
Allele	GB:AB009589	361	10596	10596	A>G	
	source	wetSNP	GB:AB009589.v10596.A>G			
	consequence	GB:AB009589_1258977CD1	362	Intron		
Allele	GB:AB009589	361	11552	11552	A>G	
	source	isSNP	SNP00023660			
	consequence	GB:AB009589_1258977CD1	362	3'		
Allele	GB:AB009589	361	11654	11654	G>T	
	source	isSNP	SNP00106046			
	consequence	GB:AB009589_1258977CD1	362	3'		

GIF OMD-genomic-fwd.gif

PD6CD6IP

Full name : programmed cell death 6-interacting protein

TABLE 1 (Cont.)

Link : PDCD6IP\_link\_cdna

Subsequence	GB:AF151793	1	3221	#364			
CDS	GB:AF151793.1	2607 bp		#365			
ORF	127	2733					
Allele	GB:AF151793	364	1051	1051	A>G		
	source	isSNP	SNP00029958				
	consequence	GB:AF151793.1	365	Missense	309-309	T>A	
Allele	GB:AF151793	364	1258	1258	A>G		
	source	isSNP	SNP00108790				
	consequence	GB:AF151793.1	365	Missense	378-378	V>I	
Allele	GB:AF151793	364	1298	1298	G>T		
	source	isSNP	SNP00108791				
	consequence	GB:AF151793.1	365	Missense	391-391	L>W	
Allele	GB:AF151793	364	1695	1695	A>G		
	source	isSNP	SNP00093444				
	consequence	GB:AF151793.1	365	Silent	523-523	L	
Allele	GB:AF151793	364	2230	2230	A>G		
	source	isSNP	SNP00121559				
	consequence	GB:AF151793.1	365	Missense	702-702	R>G	
Allele	GB:AF151793	364	2315	2315	A>G		
	source	isSNP	SNP00006604				
	consequence	GB:AF151793.1	365	Missense	730-730	L>S	
Allele	GB:AF151793	364	2386	2386	A>G		
	source	isSNP	SNP00029960				
	consequence	GB:AF151793.1	365	Missense	754-754	P>S	
Allele	GB:AF151793	364	2421	2421	A>G		
	source	isSNP	SNP00121560				
	consequence	GB:AF151793.1	365	Silent	765-765	A	
GIF	PDCD6IP-cdna-fwd.gif						

PDNP1

Full name : phosphodiesterase I (nucleotide pyrophosphatase I (homologous to mouse Ly-41 antigen))

Link : PDNP1\_link\_cdna

Subsequence	EM:HSAUTOTAX	1	3231	#366			
CDS	EM:HSAUTOTAX.2	2748 bp		#367			
ORF	50	2797					
Allele	EM:HSAUTOTAX	366	342	342	A>G		
	source	isSNP	SNP00025434				
	consequence	EM:HSAUTOTAX.2	367	Missense	98-98	A>V	
Allele	EM:HSAUTOTAX	366	696	696	A>G		
	source	isSNP	SNP00075872				
	consequence	EM:HSAUTOTAX.2	367	Missense	216-216	T>I	
Allele	EM:HSAUTOTAX	366	1682	1682	A>G		
	source	isSNP	SNP00025435				
	consequence	EM:HSAUTOTAX.2	367	Missense	545-545	P>S	
Allele	EM:HSAUTOTAX	366	1789	1789	A>G		
	source	isSNP	SNP00004604				
	consequence	EM:HSAUTOTAX.2	367	Silent	580-580	H	
Allele	EM:HSAUTOTAX	366	2398	2398	G>T		
	source	isSNP	SNP00122211				
	consequence	EM:HSAUTOTAX.2	367	Silent	783-783	V	
Allele	EM:HSAUTOTAX	366	2539	2539	A>G		
		238					

TABLE 1 (Cont.)

	source	isSNP	SNP00004605				
	consequence	EM:HSAUTOTAX.2	367	Silent	830-830	F	
Allele	EM:HSAUTOTAX	366 2681	2681	G>T			
	source	isSNP	SNP00059344				
	consequence	EM:HSAUTOTAX.2	367	Silent	878-878	R	
GIF PDNP1-cdna-fwd.gif							
Link : PDNP1_link_genomic							
Subsequence	IN:98092911313498	4217	4948	#368			
Subsequence	IN:98061109562226435		5050	5980	#369		
Subsequence	IN:98092910591328158		3611	4115	#370		
Subsequence	IN:98092911013628201		100	699	#371		
Subsequence	IN:98092911024828217		2027	2526	#372		
Subsequence	IN:98092911044928261		3068	3509	#373		
Subsequence	IN:98092911065328292		801	1418	#374		
Subsequence	IN:98092913141116289		6183	6572	#375		
Subsequence	IN:98111010592914993		1520	1926	#376		
Subsequence	IN:98111011021915028		2628	2967	#377		
Allele	IN:98092910591328158	370	232	232	A>G		
	source	isSNP	SNP00025435				
Allele	IN:98092913141116289	375	189	189	G>T		
	source	isSNP	SNP00059344				

## PLA2G2A

Full name : phospholipase A2, group IIA

Link : PLA2G2A\_link\_cdna

Subsequence	GB:HUMRASFAB	1	854	#378		
CDS	GB:HUMRASFAB.1	435 bp		#379		
ORF	136	570				
Allele	GB:HUMRASFAB	378 267	267	A>G		
	source	isSNP	SNP00010003			
	consequence	GB:HUMRASFAB.1	379	Silent	44-44	Y
Allele	GB:HUMRASFAB	378 800	800	A>G		
	source	isSNP	SNP00021612			
	consequence	GB:HUMRASFAB.1	379	3'		

GIF PLA2G2A-cdna-fwd.gif

Link : PLA2G2A\_link\_genomic

Subsequence	PLA2G2A_cds.1	51704	48629	#380		
Subsequence	PLA2G2A_mrna_build.1		52537	48418	#381	
Subsequence	GB:AL358253_1	1	180550		#382	
Subsequence	LG:474322.13_mrna_build.1		52786	48418	#383	
Subsequence	PLA2G2A_cds.2	51704	50985	#384		
mRNA	LG:474322.13_mrna_build.1		1028 bp		5 exons	#383
exon	52786	52511				
exon	51810	51665				
exon	51455	51311				
exon	51052	50946				
exon	48771	48418				
CDS	PLA2G2A_cds.1	435 bp		4 exons	#380	
exon	51704	51665				
exon	51455	51311				
exon	51052	50946				
exon	48771	48629				
CDS	PLA2G2A_cds.2	108 bp		2 exons	#384	
			239			

TABLE 1 (Cont.)

exon	51704	51665				
exon	51052	50985				
mRNA	PLA2G2A_mrna_build.1	779 bp	5 exons		#381	
exon	52537	52511				
exon	51810	51665				
exon	51455	51311				
exon	51052	50946				
exon	48771	48418				
Allele	GB:AL358253_1	382	51364	51364	A>G	
	source	isSNP	SNP00010003			
	consequence	PLA2G2A_cds.1	380	Silent		44-44 Y
	consequence	PLA2G2A_cds.2	384	Intron		
Allele	GB:AL358253_1	382	52584	52584	C>G	
	source	isSNP	SNP00021611			
	consequence	PLA2G2A_cds.1	380	5'		
	consequence	PLA2G2A_cds.2	384	5'		
GIF PLA2G2A-genomic-rev.gif						

## PPP1R5

Full name : protein phosphatase 1, regulatory (inhibitor) subunit 5

Link : PPP1R5\_link\_cdna

Subsequence	GB:Y18207_1	1	1158	#385		
CDS	GB:Y18207_1.1	954 bp		#386		
ORF	92	1045				
Allele	GB:Y18207_1	385	571	571	A>G	
	source	isSNP	SNP00041149			
	consequence	GB:Y18207_1.1	386	Silent		160-160 E
Allele	GB:Y18207_1	385	1096	1096	G>T	
	source	isSNP	SNP00060710			
	consequence	GB:Y18207_1.1	386	3'		

GIF PPP1R5-cdna-fwd.gif

Link : PPP1R5\_link\_genomic

Subsequence	GB:AC020691_2	1	152048	#387		
Subsequence	PPP1R5_mrna_build.1		103997	107245	#388	
Subsequence	PPP1R5_cds.1	106194	107132	#389		
CDS	PPP1R5_cds.1	939 bp	1 exon	#389		
exon	106194	107132				
mRNA	PPP1R5_mrna_build.1	1160 bp	2 exons	#388		
exon	103997	104103				
exon	106193	107245				
Allele	GB:AC020691_2	387	106523	106523	G>T	
	source	wetSNP	GB:AC020691_2.v106523.T>G			
	consequence	PPP1R5_cds.1	389	Missense		110-110 D>E
Allele	GB:AC020691_2	387	106658	106658	A>G	
	source	isSNP	SNP00041149			
	consequence	PPP1R5_cds.1	389	Silent		155-155 E
Allele	GB:AC020691_2	387	107183	107183	G>T	
	source	isSNP	SNP00060710			
	consequence	PPP1R5_cds.1	389	3'		

GIF PPP1R5-genomic-fwd.gif

TABLE 1 (Cont.)

## PRELP

Full name : proline arginine-rich end leucine-rich repeat protein

Link : PRELP\_link\_cdna

Subsequence	GB:HSU29089	1	1560	#390		
CDS	GB:HSU29089.1	1149 bp		#391		
ORF	129	1277				
Allele	GB:HSU29089	390	1170	1170	G>T	
	source	isSNP	SNP00001359			
	consequence	GB:HSU29089.1	391	Missense	348-348	N>H
Allele	GB:HSU29089	390	1489	1489	G>T	
	source	isSNP	SNP00001361			
	consequence	GB:HSU29089.1	391	3'		

GIF PRELP-cdna-fwd.gif

Link : PRELP\_link\_genomic

Subsequence	PRELP_cds.1	82496	86192	#392		
Subsequence	GB:AC022000_1	1	154681	#393		
Subsequence	PRELP_mrna_build.1		75139	86474	#394	
CDS	PRELP_cds.1	1149 bp	2 exons	#392		
exon	82496	83468				
exon	86017	86192				
mRNA	PRELP_mrna_build.1		1559 bp	3 exons	#394	
exon	75139	75250				
exon	82480	83468				
exon	86017	86474				
Allele	GB:AC022000_1	393	86085	86085	G>T	
	source	isSNP	SNP00001359			
	consequence	PRELP_cds.1	392	Missense	348-348	N>H
Allele	GB:AC022000_1	393	86404	86404	G>T	
	source	isSNP	SNP00001361			
	consequence	PRELP_cds.1	392	3'		

GIF PRELP-genomic-fwd.gif

## PRSS11

Full name : serine protease.

Link : FL\_1787335\_link\_cdna

Subsequence	FN:1787335CB1	1	2054	#395		
CDS	FN:1787335CB1.1	1443 bp		#396		
ORF	49	1491				
Allele	FN:1787335CB1	395	150	150	A>G	
	source	isSNP	SNP00068999			
	consequence	FN:1787335CB1.1	396	Silent	34-34	A
Allele	FN:1787335CB1	395	156	156	G>T	
	source	isSNP	SNP00117078			
	consequence	FN:1787335CB1.1	396	Silent	36-36	G
Allele	FN:1787335CB1	395	914	914	A>G	
	source	isSNP	SNP00120314			
	consequence	FN:1787335CB1.1	396	Missense	289-289	Q>R
Allele	FN:1787335CB1	395	1321	1321	C>G	
	source	isSNP	SNP00105589			
	consequence	FN:1787335CB1.1	396	Missense	425-425	A>P
Allele	FN:1787335CB1	395	1521	1521	A>G	
	source	isSNP	SNP00105590			
	consequence	FN:1787335CB1.1	396	3'		

TABLE 1 (Cont.)

GIF PRSS11-cdna-fwd.gif						
Link : FL_1787335_link_genomic						
Subsequence	GB:AF157623_1_1787335CD1	17526	70213	#397		
Subsequence	GB:AF157623_1	1	79597	#398		
Subsequence	FL_1787335_mrna_build.1	17478	70761	#399		
CDS	GB:AF157623_1_1787335CD1	1443 bp	9 exons	#397		
exon	17526	17997				
exon	44770	44869				
exon	45290	45494				
exon	62561	62755				
exon	63240	63272				
exon	64526	64640				
exon	65966	66023				
exon	67827	67922				
exon	70045	70213				
mRNA	FL_1787335_mrna_build.1	2039 bp	9 exons	#399		
exon	17478	17997				
exon	44770	44869				
exon	45290	45494				
exon	62561	62755				
exon	63240	63272				
exon	64526	64640				
exon	65966	66023				
exon	67827	67922				
exon	70045	70761				
Allele	GB:AF157623_1	398	17627	17627	A>G	
	source	isSNP	SNP00068999			
	consequence	GB:AF157623_1_1787335CD1	397		Silent	
34-34 A						
Allele	GB:AF157623_1	398	17633	17633	G>T	
	source	isSNP	SNP00117078			
	consequence	GB:AF157623_1_1787335CD1	397		Silent	
36-36 G						
Allele	GB:AF157623_1	398	21721	21721	A>G	
	source	isSNP	SNP00101582			
	consequence	GB:AF157623_1_1787335CD1	397		Intron	
Allele	GB:AF157623_1	398	35790	35790	A>G	
	source	isSNP	SNP00049308			
	consequence	GB:AF157623_1_1787335CD1	397		Intron	
Allele	GB:AF157623_1	398	44762	44762	G>T	
	source	wetSNP	GB:AF157623_1.v44762.G>T			
	consequence	GB:AF157623_1_1787335CD1	397		Intron	
Allele	GB:AF157623_1	398	45470	45470	A>G	
	source	wetSNP	GB:AF157623_1.v45470.C>T			
	consequence	GB:AF157623_1_1787335CD1	397		Silent	
251-251	I					
Allele	GB:AF157623_1	398	45587	45587	A>G	
	source	wetSNP	GB:AF157623_1.v45587.C>T			
	consequence	GB:AF157623_1_1787335CD1	397		Intron	
Allele	GB:AF157623_1	398	47792	47792	A>G	
	source	isSNP	SNP00105588			
	consequence	GB:AF157623_1_1787335CD1	397		Intron	
Allele	GB:AF157623_1	398	47834	47834	A>G	
	source	isSNP	SNP00120312			
	consequence	GB:AF157623_1_1787335CD1	397		Intron	



TABLE 1 (Cont.)

Allele	GB:AF157623_1	398	47913	47913	A>G	
	source	isSNP	SNP00120313			
	consequence	GB:AF157623_1_1787335CD1		397	Intron	
Allele	GB:AF157623_1	398	62541	62541	A>G	
	source	wetSNP	GB:AF157623_1.v62541.G>A			
	consequence	GB:AF157623_1_1787335CD1		397	Intron	
Allele	GB:AF157623_1	398	62545	62545	A>G	
	source	wetSNP	GB:AF157623_1.v62545.G>A			
	consequence	GB:AF157623_1_1787335CD1		397	Intron	
Allele	GB:AF157623_1	398	62649	62649	A>G	
	source	isSNP	SNP00120314			
	consequence	GB:AF157623_1_1787335CD1		397	Missense	
289-289	Q>R					
Allele	GB:AF157623_1	398	63355	63360	TGTTTT>TT	
	source	wetSNP	GB:AF157623_1.v63355.TGTTTT>TT			
	consequence	GB:AF157623_1_1787335CD1		397	Intron	
Allele	GB:AF157623_1	398	70243	70243	A>G	
	source	isSNP	SNP00105590			
	consequence	GB:AF157623_1_1787335CD1		397	3'	
GIF PRSS11-genomic-fwd.gif						

## PTGS2

Full name : Prostaglandin-endoperoxide Synthase 2

Link : PTGS2\_link\_cdna

Subsequence	EM:HSCYCLOX	1	3387	#400
Allele	EM:HSCYCLOX	400	403	C>G
	source	isSNP	SNP00046167	
Allele	EM:HSCYCLOX	400	880	G>T
	source	isSNP	SNP00076329	
Allele	EM:HSCYCLOX	400	2033	A>G
	source	isSNP	SNP00076330	
Allele	EM:HSCYCLOX	400	2300	A>G
	source	isSNP	SNP00046168	
Allele	EM:HSCYCLOX	400	2983	A>G
	source	isSNP	SNP00046169	

Link : PTGS2\_link\_genomic

Subsequence	GB:HUMPTGS2	101	11097	#401		
Subsequence	PTGS2_cds.1	1925	8146	#402		
Subsequence	PTGS2_mrna_build.1		1828	9607	#403	
CDS	PTGS2_cds.1	1815 bp	10 exons	#402		
	exon	1925	1976			
	exon	2777	2893			
	exon	3014	3157			
	exon	3811	3954			
	exon	4670	4851			
	exon	5584	5667			
	exon	5787	6033			
	exon	6315	6601			
	exon	7103	7250			
	exon	7737	8146			
mRNA	PTGS2_mrna_build.1		3373 bp	10 exons	#403	
	exon	1828	1976			
	exon	2777	2893			

TABLE 1 (Cont.)

exon	3014	3157				
exon	3811	3954				
exon	4670	4851				
exon	5584	5667				
exon	5787	6033				
exon	6315	6601				
exon	7103	7250				
exon	7737	9607				
Allele	GB:HUMPTGS2	401	3050	3050	C>G	
	source	wetSNP		GB:HUMPTGS2.v3050.G>C		
	consequence	PTGS2_cds.1	402	Silent	102-102	V
Allele	GB:HUMPTGS2	401	3090	3090	A>G	
	source	wetSNP		GB:HUMPTGS2.v3090.C>T		
	consequence	PTGS2_cds.1	402	Intron		
Allele	GB:HUMPTGS2	401	3174	3174	C>G	
	source	wetSNP		GB:HUMPTGS2.v3174.G>C		
	consequence	PTGS2_cds.1	402	Intron		
Allele	GB:HUMPTGS2	401	3793	3793	A>G	
	source	wetSNP		GB:HUMPTGS2.v3793.C>T		
	consequence	PTGS2_cds.1	402	Silent	132-132	S
Allele	GB:HUMPTGS2	401	3829	3829	A>G	
	source	wetSNP		GB:HUMPTGS2.v3829.T>C		
	consequence	PTGS2_cds.1	402	Silent	144-144	D
Allele	GB:HUMPTGS2	401	5605	5605	A>G	
	source	wetSNP		GB:HUMPTGS2.v5605.G>A		
	consequence	PTGS2_cds.1	402	Intron		
Allele	GB:HUMPTGS2	401	5676	5681	TATTTT>TT	
	source	wetSNP		GB:HUMPTGS2.v5676.TATTTT>TT		
	consequence	PTGS2_cds.1	402	Intron		
Allele	GB:HUMPTGS2	401	5746	5746	G>T	
	source	isSNP	SNP00076329			
	consequence	PTGS2_cds.1	402	Stop	261-261	
Allele	GB:HUMPTGS2	401	6249	6249	A>G	
	source	wetSNP		GB:HUMPTGS2.v6249.G>A		
	consequence	PTGS2_cds.1	402	Silent	335-335	V
Allele	GB:HUMPTGS2	401	6444	6444	A>G	
	source	wetSNP		GB:HUMPTGS2.v6444.G>A		
	consequence	PTGS2_cds.1	402	Silent	400-400	L
Allele	GB:HUMPTGS2	401	6453	6453	A>G	
	source	wetSNP		GB:HUMPTGS2.v6453.T>C		
	consequence	PTGS2_cds.1	402	Silent	403-403	H
Allele	GB:HUMPTGS2	401	7581	7581	A>G	
	source	wetSNP		GB:HUMPTGS2.v7581.T>C		
	consequence	PTGS2_cds.1	402	Intron		
Allele	GB:HUMPTGS2	401	7763	7763	A>G	
	source	wetSNP		GB:HUMPTGS2.v7763.T>C		
	consequence	PTGS2_cds.1	402	Missense	511-511	V>A
Allele	GB:HUMPTGS2	401	7986	7986	G>T	
	source	wetSNP		GB:HUMPTGS2.v7986.C>A		
	consequence	PTGS2_cds.1	402	Silent	585-585	R
Allele	GB:HUMPTGS2	401	8167	8167	A>G	
	source	isSNP	SNP00076330			
	consequence	PTGS2_cds.1	402	3'		
Allele	GB:HUMPTGS2	401	8434	8434	A>G	
	source	isSNP	SNP00046168			

TABLE 1 (Cont.)

consequence PTGS2\_cds.1 402 3'  
 Allele GB:HUMPTGS2 401 8473 8473 A>G  
 source isSNP SNP00012871  
 consequence PTGS2\_cds.1 402 3'  
 Allele GB:HUMPTGS2 401 9102 9102 A>G  
 source isSNP SNP00046169  
 consequence PTGS2\_cds.1 402 3'  
 GIF PTGS2-genomic-fwd.gif

## PTHLH

Full name : PTHLH

Link : PTHLH\_link\_genomic

Subsequence PTHLH\_cds.1 106964 117899 #404  
 Subsequence GB:AC008011\_6 1 183178 #405  
 Subsequence PTHLH\_mrna\_build.1 106942 118367 #406  
 CDS PTHLH\_cds.1 534 bp 3 exons #404  
 exon 106964 107064  
 exon 112688 113110  
 exon 117890 117899  
 mRNA PTHLH\_mrna\_build.1 1024 bp 3 exons #406  
 exon 106942 107064  
 exon 112688 113110  
 exon 117890 118367  
 Allele GB:AC008011\_6 405 113450 113450 A>G  
 source isSNP SNP00043978  
 consequence PTHLH\_cds.1 404 Intron  
 Allele GB:AC008011\_6 405 115075 115075 A>G  
 source dbSNP gn1|dbSNP|ss1455356\_allele  
 consequence PTHLH\_cds.1 404 Intron  
 Allele GB:AC008011\_6 405 115160 115160 A>G  
 source dbSNP gn1|dbSNP|ss1067559\_allele  
 consequence PTHLH\_cds.1 404 Intron  
 GIF PTHLH-genomic-fwd.gif

## PTHR1

Full name : PTHR1

Link : PTHR1\_link\_cdna

Subsequence GB:HUMPTHR 1 1948 #407  
 CDS GB:HUMPTHR.1 1782 bp #408  
 ORF 29 1810  
 Allele GB:HUMPTHR 407 1417 1417 A>G  
 source isSNP SNP00007059  
 consequence GB:HUMPTHR.1 408 Silent 463-463 N

GIF PTHR1-cdna-fwd.gif

Link : PTHR1\_link\_genomic

Subsequence GB:HSPTHPRH1 1 262 #409  
 Subsequence GB:HSPTHPRH2 363 769 #410  
 Subsequence GB:HSPTHPRH3 870 1168 #411  
 Subsequence GB:HSPTHPRH4 1269 2146 #412  
 Subsequence GB:HSPTHPRH5 2247 3249 #413  
 Subsequence GB:HSPTHPRH6 3350 4062 #414  
 245

TABLE 1 (Cont.)

Subsequence	GB:HSPTHPRH7	4163	4475	#415	
Subsequence	GB:HSPTHPRH8	4576	4995	#416	
Subsequence	GB:HSPTHPRH9	5096	5696	#417	
Subsequence	PTHR1_cds.1 107	5558	#418		
Subsequence	PTHR1_mrna_build.1	79	5696	#419	
CDS	PTHR1_cds.1	1782 bp	14 exons	#418	
exon	107	181			
exon	456	558			
exon	936	1070			
exon	1436	1546			
exon	1655	1773			
exon	1959	2053			
exon	2351	2546			
exon	2980	3133			
exon	3547	3607			
exon	3938	4004			
exon	4273	4367			
exon	4628	4769			
exon	4851	4892			
exon	5172	5558			
mRNA	PTHR1_mrna_build.1	1948 bp	14 exons	#419	
exon	79	181			
exon	456	558			
exon	936	1070			
exon	1436	1546			
exon	1655	1773			
exon	1959	2053			
exon	2351	2546			
exon	2980	3133			
exon	3547	3607			
exon	3938	4004			
exon	4273	4367			
exon	4628	4769			
exon	4851	4892			
exon	5172	5696			
Allele	GB:HSPTHPRH3	411	104	104	A>G
source	wetSNP				GB:HSPTHPRH3.v104.G>A
consequence	PTHR1_cds.1	418	Silent		72-72 A
Allele	GB:HSPTHPRH8	416	311	311	A>G
source	wetSNP				GB:HSPTHPRH8.v311.T>C
consequence	PTHR1_cds.1	418	Silent		463-463 N
GIF	PTHR1-genomic-fwd.gif				

RARA

Full name : retinoic acid receptor, alpha

Link : RARA\_link\_cdna

Subsequence	GB:NM_000964	1	2907	#420	
CDS	GB:NM_000964.1	1389 bp		#421	
ORF	103	1491			
Allele	GB:NM_000964	420	2327	2327	A>G
source	isSNP	SNP00016145			
consequence	GB:NM_000964.1	421	3'		
Allele	GB:NM_000964	420	2439	2439	A>G
		246			

TABLE 1 (Cont.)

source isSNP SNP00049381  
 consequence GB:NM\_000964.1 421 3'  
 GIF RARA-cdna-fwd.gif

## RIN1

Full name : ras inhibitor

Link : RIN1\_link\_cdna

Subsequence	GB:HUMRASINF	1	1285	#422
Allele	GB:HUMRASINF	422 260	260	A>G
	source isSNP	SNP00123606		
Allele	GB:HUMRASINF	422 424	424	A>G
	source isSNP	SNP00123607		
Allele	GB:HUMRASINF	422 722	722	A>G
	source isSNP	SNP00033587		
Allele	GB:HUMRASINF	422 921	921	A>G
	source isSNP	SNP00007808		

## ROR2

Full name : receptor tyrosine kinase-like orphan receptor 2

Link : ROR2\_link\_cdna

Subsequence	GB:NM_004560	1	4092	#423	
CDS	GB:NM_004560.1	2832 bp		#424	
ORF	200 3031				
Allele	GB:NM_004560	423 932	932	A>G	
	source isSNP	SNP00098926			
	consequence	GB:NM_004560.1	424	Missense	245-245 A>T
Allele	GB:NM_004560	423 1460	1460	A>G	
	source isSNP	SNP00098927			
	consequence	GB:NM_004560.1	424	Missense	421-421 L>F
Allele	GB:NM_004560	423 1973	1973	A>G	
	source isSNP	SNP00098928			
	consequence	GB:NM_004560.1	424	Missense	592-592 F>L
Allele	GB:NM_004560	423 2287	2287	A>G	
	source isSNP	SNP00028168			
	consequence	GB:NM_004560.1	424	Silent	696-696 Y
Allele	GB:NM_004560	423 2353	2353	A>G	
	source isSNP	SNP00098929			
	consequence	GB:NM_004560.1	424	Silent	718-718 P
Allele	GB:NM_004560	423 2654	2654	A>G	
	source isSNP	SNP00028169			
	consequence	GB:NM_004560.1	424	Missense	819-819 V>I
Allele	GB:NM_004560	423 3743	3743	A>G	
	source isSNP	SNP00028170			
	consequence	GB:NM_004560.1	424	3'	
Allele	GB:NM_004560	423 3872	3872	G>T	
	source isSNP	SNP00074568			
	consequence	GB:NM_004560.1	424	3'	
Allele	GB:NM_004560	423 3919	3919	G>T	
	source isSNP	SNP00074569			
	consequence	GB:NM_004560.1	424	3'	

GIF ROR2-cdna-fwd.gif

TABLE 1 (Cont.)

## RORA

Full name : RAR-related orphan receptor alpha

Link : RORA\_link\_genomic

Subsequence	RORA_cds.1	64220	3076	#425	
Subsequence	RORA_cds.2	64220	3076	#426	
Subsequence	RORA_cds.4	64220	3076	#427	
Subsequence	GB:AC012344_4_000018		1	9454	#428
Subsequence	GB:AC012344_4_000020		9555	21185	#429
Subsequence	GB:AC012344_4_000021		21286	34347	#430
Subsequence	GB:AC012344_4_000019		34448	43824	#431
Subsequence	GB:AC012344_4_000023		43925	65900	#432
Subsequence	RORA_mrna_build.1	64309	2885		#433
Subsequence	RORA_mrna_build.4	64290	2885		#434
mRNA	RORA_mrna_build.4	1908 bp		11 exons	#434
exon	64290	64084			
exon	51847	51714			
exon	25290	25205			
exon	19553	19412			
exon	16417	16022			
exon	10425	10304			
exon	9288	9156			
exon	8488	8381			
exon	6690	6580			
exon	5625	5513			
exon	3240	2885			
CDS	RORA_cds.1	1671 bp	12 exons		#425
exon	64220	64084			
exon	43229	43148			
exon	41851	41776			
exon	25290	25205			
exon	19553	19412			
exon	16417	16022			
exon	10425	10304			
exon	9288	9156			
exon	8488	8381			
exon	6690	6580			
exon	5625	5513			
exon	3240	3076			
CDS	RORA_cds.2	1275 bp	11 exons		#426
exon	64220	64084			
exon	43229	43148			
exon	41851	41776			
exon	25290	25205			
exon	19553	19412			
exon	10425	10304			
exon	9288	9156			
exon	8488	8381			
exon	6690	6580			
exon	5625	5513			
exon	3240	3076			
mRNA	RORA_mrna_build.1	1951 bp	12 exons		#433
exon	64309	64084			
exon	43229	43148			

TABLE 1 (Cont.)

exon	41851	41776		
exon	25290	25205		
exon	19553	19412		
exon	16417	16022		
exon	10425	10304		
exon	9288	9156		
exon	8488	8381		
exon	6690	6580		
exon	5625	5513		
exon	3240	2885		
CDS	RORA_cds.4	1647 bp	11 exons	#427
exon	64220	64084		
exon	51847	51714		
exon	25290	25205		
exon	19553	19412		
exon	16417	16022		
exon	10425	10304		
exon	9288	9156		
exon	8488	8381		
exon	6690	6580		
exon	5625	5513		
exon	3240	3076		
Allele	GB:AC012344_4_000020	429	11153	11153 A>G
	source	dbSNP gnl dbSNP ss380580_allele		
	consequence	RORA_cds.1 425	Intron	
	consequence	RORA_cds.2 426	Intron	
	consequence	RORA_cds.4 427	Intron	
Allele	GB:AC012344_4_000020	429	11182	11182 A>G
	source	dbSNP gnl dbSNP ss380580_allele		
	consequence	RORA_cds.1 425	Intron	
	consequence	RORA_cds.2 426	Intron	
	consequence	RORA_cds.4 427	Intron	
Allele	GB:AC012344_4_000020	429	11183	11183 A>T
	source	dbSNP gnl dbSNP ss507731_allele		
	consequence	RORA_cds.1 425	Intron	
	consequence	RORA_cds.2 426	Intron	
	consequence	RORA_cds.4 427	Intron	
Allele	GB:AC012344_4_000020	429	11254	11254 A>G
	source	dbSNP gnl dbSNP ss380580_allele		
	consequence	RORA_cds.1 425	Intron	
	consequence	RORA_cds.2 426	Intron	
	consequence	RORA_cds.4 427	Intron	
Allele	GB:AC012344_4_000020	429	11255	11255 A>T
	source	dbSNP gnl dbSNP ss507731_allele		
	consequence	RORA_cds.1 425	Intron	
	consequence	RORA_cds.2 426	Intron	
	consequence	RORA_cds.4 427	Intron	
Allele	GB:AC012344_4_000020	429	11264	11264 A>G
	source	dbSNP gnl dbSNP ss380580_allele		
	consequence	RORA_cds.1 425	Intron	
	consequence	RORA_cds.2 426	Intron	
	consequence	RORA_cds.4 427	Intron	
Allele	GB:AC012344_4_000020	429	11265	11265 A>T
	source	dbSNP gnl dbSNP ss507731_allele		
	consequence	RORA_cds.1 425	Intron	

TABLE 1 (Cont.)

	consequence	RORA_cds.2	426	Intron	
	consequence	RORA_cds.4	427	Intron	
Allele	GB:AC012344_4_000020	429	11320	11320	A>G
	source	dbSNP gnl dbSNP ss380580_allele			
	consequence	RORA_cds.1	425	Intron	
	consequence	RORA_cds.2	426	Intron	
	consequence	RORA_cds.4	427	Intron	
GIF RORA-genomic-rev.gif					

## SCRG1

Full name : scrapie responsive protein

Link : SCRG1\_link\_genomic

Subsequence	SCRG1_cds.1	30577	33650	#435	
Subsequence	GB:AC009588_4	1	164772		#436
Subsequence	SCRG1_mrna_build.1		30561	33845	#437
CDS	SCRG1_cds.1	297 bp	2 exons	#435	
exon	30577	30818			
exon	33596	33650			
mRNA	SCRG1_mrna_build.1		508 bp	2 exons	#437
exon	30561	30818			
exon	33596	33845			
GIF SCRG1-genomic-fwd.gif					

## SCYA20

Full name : small inducible cytokine subfamily A member 20

Link : SCYA20\_link\_cdna

Subsequence	GB:HSU64197	1	821	#438	
CDS	GB:HSU64197.1	288 bp		#439	
ORF	43	330			
Allele	GB:HSU64197	438	341	341	A>G
	source	isSNP	SNP00037526		
	consequence	GB:HSU64197.1	439	3'	
Allele	GB:HSU64197	438	728	728	A>G
	source	isSNP	SNP00037527		
	consequence	GB:HSU64197.1	439	3'	

GIF SCYA20-cdna-fwd.gif

Link : SCYA20\_link\_genomic

Subsequence	SCYA20_cds.1	73925	77096	#440	
Subsequence	GB:AC027560_2	1	129588		#441
Subsequence	SCYA20_mrna_build.1		73883	77577	#442
CDS	SCYA20_cds.1	288 bp	4 exons	#440	
exon	73925	74000			
exon	75470	75581			
exon	76320	76397			
exon	77075	77096			
mRNA	SCYA20_mrna_build.1		811 bp	4 exons	#442
exon	73883	74000			
exon	75470	75581			
exon	76320	76397			
exon	77075	77577			
Allele	GB:AC027560_2	441	77107	77107	A>G



TABLE 1 (Cont.)

	source	isSNP	SNP00037526		
	consequence	SCYA20_cds.1	440	3'	
Allele	GB:AC027560_2	441	77493	77493	A>G
	source	isSNP	SNP00037527		
	consequence	SCYA20_cds.1	440	3'	
GIF	SCYA20-genomic-fwd.gif				

## SDC2

Full name : syndecan 2

Link : SDC2\_link\_cdna

Subsequence	GB:HUMHSPGC	1	3414	#443		
CDS	GB:HUMHSPGC.2	1194	bp	#444		
ORF	1	1194				
Allele	GB:HUMHSPGC	443	435	435	A>G	
	source	isSNP	SNP00116695			
	consequence	GB:HUMHSPGC.2	444	Silent	145-145	P
Allele	GB:HUMHSPGC	443	463	463	C>G	
	source	isSNP	SNP00050825			
	consequence	GB:HUMHSPGC.2	444	Missense	155-155	L>V
Allele	GB:HUMHSPGC	443	741	741	A>G	
	source	isSNP	SNP00033651			
	consequence	GB:HUMHSPGC.2	444	Silent	247-247	Y
Allele	GB:HUMHSPGC	443	1041	1041	G>T	
	source	isSNP	SNP00099428			
	consequence	GB:HUMHSPGC.2	444	Silent	347-347	A
GIF	SDC2-cdna-fwd.gif					

## SDC4

Full name : syndecan 4

Link : FL\_1394592\_link\_cdna

Subsequence	FN:1394592CB1	1	2112	#445	
CDS	FN:1394592CB1.1	594	bp	#446	
ORF	23	616			
CDS	GB:HS453C12_1394592CD1	594	bp	#272	
ORF	87967	88026			
ORF	100431	100569			
ORF	103282	103328			
ORF	105787	105985			
ORF	108936	109084			
mRNA	FL_1394592_mrna_build.1	2110	bp	#274	
ORF	87945	88026			
ORF	100431	100569			
ORF	103282	103328			
ORF	105787	105985			
ORF	108936	110578			
Allele	FN:1394592CB1	445	653	653	C>G
	source	isSNP	SNP00124074		
	consequence	FN:1394592CB1.1	446	3'	
Allele	FN:1394592CB1	445	749	749	A>G
	source	isSNP	SNP00124075		
	consequence	FN:1394592CB1.1	446	3'	

TABLE 1 (Cont.)

Allele	FN:1394592CB1	445	856	856	A>G
	source	isSNP	SNP00053065		
	consequence	FN:1394592CB1.1	446	3'	
Allele	FN:1394592CB1	445	884	884	A>G
	source	isSNP	SNP00066145		
	consequence	FN:1394592CB1.1	446	3'	
Allele	FN:1394592CB1	445	1048	1048	A>G
	source	isSNP	SNP00066146		
	consequence	FN:1394592CB1.1	446	3'	
Allele	FN:1394592CB1	445	1214	1214	A>G
	source	isSNP	SNP00029910		
	consequence	FN:1394592CB1.1	446	3'	
GIF SDC4-cdna-fwd.gif					
Link : FL_1250708_link_genomic					
Subsequence	GB:HS453C12	1	147620	#271	
Subsequence	GB:HS453C12_1394592CD1		87967	109084	#272
Subsequence	GB:HS453C12_2027624CD1		20194	10528	#273
Subsequence	FL_1394592_mrna_build.1		87945	110578	#274
Subsequence	FL_2027624_mrna_build.1		20197	6152	#275
Subsequence	OA21_cds.1		20194	17050	#276
CDS	GB:HS453C12_1394592CD1	594 bp		5 exons	#272
exon	87967	88026			
exon	100431	100569			
exon	103282	103328			
exon	105787	105985			
exon	108936	109084			
mRNA	FL_1394592_mrna_build.1	2110 bp		5 exons	#274
exon	87945	88026			
exon	100431	100569			
exon	103282	103328			
exon	105787	105985			
exon	108936	110578			
Allele	GB:HS453C12	271	90320	90320	A>G
	source	isSNP	SNP00026142		
	consequence	GB:HS453C12_1394592CD1	272	Intron	
Allele	GB:HS453C12	271	90420	90420	C>G
	source	isSNP	SNP00026143		
	consequence	GB:HS453C12_1394592CD1	272	Intron	
Allele	GB:HS453C12	271	96768	96768	A>G
	source	dbSNP gnl dbSNP ss736312_allele			
	consequence	GB:HS453C12_1394592CD1	272	Intron	
Allele	GB:HS453C12	271	109121	109121	C>G
	source	isSNP	SNP00124074		
	consequence	GB:HS453C12_1394592CD1	272	3'	
Allele	GB:HS453C12	271	109217	109217	A>G
	source	isSNP	SNP00124075		
	consequence	GB:HS453C12_1394592CD1	272	3'	
Allele	GB:HS453C12	271	109324	109324	A>G
	source	isSNP	SNP00053065		
	consequence	GB:HS453C12_1394592CD1	272	3'	
Allele	GB:HS453C12	271	109352	109352	A>G
	source	isSNP	SNP00066145		
	consequence	GB:HS453C12_1394592CD1	272	3'	
Allele	GB:HS453C12	271	109516	109516	A>G
	source	isSNP	SNP00066146		

TABLE 1 (Cont.)

consequence GB:HS453C12\_1394592CD1 272 3'  
 Allele GB:HS453C12 271 109682 109682 A>G  
 source isSNP SNP00029910  
 consequence GB:HS453C12\_1394592CD1 272 3'  
 GIF SDC4-genomic-fwd.gif

## SEDL

Full name : sedlin

Link : SEDL\_link\_cdna

Subsequence GB:NМ\_014563\_1 1 2816 #447  
 CDS GB:NМ\_014563\_1.1 423 bp #448  
 ORF 230 652  
 Allele GB:NМ\_014563\_1 447 991 991 G>T  
 source dbSNP gn1|dbSNP|ss380525\_allele  
 source dbSNP gn1|dbSNP|ss531221\_allele  
 consequence GB:NМ\_014563\_1.1 448 3'  
 Allele GB:NМ\_014563\_1 447 2026 2026 A>G  
 source dbSNP gn1|dbSNP|ss637643\_allele  
 source dbSNP gn1|dbSNP|ss869682\_allele  
 source dbSNP gn1|dbSNP|ss1272499\_allele  
 source dbSNP gn1|dbSNP|ss232503\_allele  
 source dbSNP gn1|dbSNP|ss459122\_allele  
 consequence GB:NМ\_014563\_1.1 448 3'  
 Allele GB:NМ\_014563\_1 447 2391 2391 C>G  
 source isSNP SNP00010387  
 consequence GB:NМ\_014563\_1.1 448 3'  
 GIF SEDL-cdna-fwd.gif

## SKI

Full name : v-ski avian sarcoma viral oncogene homolog

Link : SKI\_link\_cdna

Subsequence GB:NМ\_003036 1 3511 #449  
 CDS GB:NМ\_003036.1 2187 bp #450  
 ORF 73 2259  
 Allele GB:NМ\_003036 449 528 528 A>G  
 source isSNP SNP00068450  
 consequence GB:NМ\_003036.1 450 Silent 152-152 R  
 Allele GB:NМ\_003036 449 1146 1146 A>G  
 source isSNP SNP00068451  
 consequence GB:NМ\_003036.1 450 Silent 358-358 T  
 Allele GB:NМ\_003036 449 3482 3482 C>G  
 source isSNP SNP00068452  
 consequence GB:NМ\_003036.1 450 3'  
 GIF SKI-cdna-fwd.gif

## SOD2

Full name : superoxide dismutase 2, mitochondrial

Link : SOD2\_link\_cdna

Subsequence EM:HSSOD 1 1026 #451  
 253

TABLE 1 (Cont.)

Allele	EM:HSSOD	451	243	243	A>G	
	source	isSNP	SNP00021476			
Link : SOD2_link_genomic						
Subsequence	EM:S77127	101	12957	#452		
Subsequence	SOD2_link_cds.1	957	11597	#453		
Subsequence	SOD2_mrna_build.1	953	11950	#454		
mRNA	SOD2_mrna_build.1	1026 bp	5 exons	#454		
exon	953	979				
exon	1260	1462				
exon	5859	5975				
exon	9061	9240				
exon	11452	11950				
CDS	SOD2_link_cds.1	669 bp	5 exons	#453		
exon	957	979				
exon	1260	1462				
exon	5859	5975				
exon	9061	9240				
exon	11452	11597				
Allele	EM:S77127	452	1183	1183	A>G	
	source	isSNP	SNP00003080			
	source	wetSNP	EM:S77127.v1183.C>T			
	consequence	SOD2_link_cds.1	453	Missense	16-16	A>V
Allele	EM:S77127	452	1456	1456	G>T	
	source	wetSNP	EM:S77127.v1456.A>C			
	consequence	SOD2_link_cds.1	453	Intron		
Allele	EM:S77127	452	1734	1734	A>G	
	source	isSNP	SNP00107369			
	consequence	SOD2_link_cds.1	453	Intron		
GIF SOD2-genomic-fwd.gif						

## SOD3

Full name : superoxide dismutase 3, extracellular

Link : SOD3\_link\_cdna

Subsequence	GB:SOD3	1	1984	#455		
CDS	GB:SOD3.1	723 bp		#456		
ORF	664	1386				
Allele	GB:SOD3	455	835	835	A>G	
	source	isSNP	SNP00033027			
	consequence	GB:SOD3.1	456	Missense	58-58	T>A
Allele	GB:SOD3	455	874	874	A>G	
	source	isSNP	SNP00062433			
	consequence	GB:SOD3.1	456	Silent	71-71	L
Allele	GB:SOD3	455	1469	1469	A>G	
	source	isSNP	SNP00067750			
	consequence	GB:SOD3.1	456	3'		
Allele	GB:SOD3	455	1496	1496	A>G	
	source	isSNP	SNP00007500			
	consequence	GB:SOD3.1	456	3'		
Allele	GB:SOD3	455	1817	1817	G>T	
	source	isSNP	SNP00104042			
	consequence	GB:SOD3.1	456	3'		
Allele	GB:SOD3	455	1826	1826	A>G	
	source	isSNP	SNP00031110			

TABLE 1 (Cont.)

	consequence	GB:SOD3.1	456	3'				
Allele	GB:SOD3	455	1932	1932	A>G			
	source	isSNP	SNP00050239					
	consequence	GB:SOD3.1	456	3'				
GIF SOD3-cdna-fwd.gif								
Link : FL_1534327_link_genomic								
Subsequence	GB:HSU10116	1	10079	#457				
Subsequence	GB:HSU10116_1534327CD1	5085	5807	#458				
Subsequence	FL_1534327_mrna_build.1	1130	6405	#459				
mRNA	FL_1534327_mrna_build.1	1427 bp	2 exons	#459				
exon	1130	1219						
exon	5069	6405						
CDS	GB:HSU10116_1534327CD1	723 bp	1 exon	#458				
exon	5085	5807						
Allele	GB:HSU10116	457	5256	5256	A>G			
	source	isSNP	SNP00033027					
	consequence	GB:HSU10116_1534327CD1	458	Missense	58-58	T>A		
Allele	GB:HSU10116	457	5295	5295	A>G			
	source	isSNP	SNP00062433					
	consequence	GB:HSU10116_1534327CD1	458	Silent	71-71	L		
Allele	GB:HSU10116	457	5890	5890	A>G			
	source	isSNP	SNP00067750					
	consequence	GB:HSU10116_1534327CD1	458	3'				
Allele	GB:HSU10116	457	5917	5917	A>G			
	source	isSNP	SNP00007500					
	consequence	GB:HSU10116_1534327CD1	458	3'				
Allele	GB:HSU10116	457	6238	6238	G>T			
	source	isSNP	SNP00104042					
	consequence	GB:HSU10116_1534327CD1	458	3'				
Allele	GB:HSU10116	457	6247	6247	A>G			
	source	isSNP	SNP00031110					
	consequence	GB:HSU10116_1534327CD1	458	3'				
Allele	GB:HSU10116	457	6353	6353	A>G			
	source	isSNP	SNP00050239					
	consequence	GB:HSU10116_1534327CD1	458	3'				
GIF SOD3-genomic-fwd.gif								

## SOX9

Full name : SOX9

Link : SOX9\_link\_cdna

Subsequence	GB:HSSOX9MRN	1	3923	#460				
CDS	GB:HSSOX9MRN.2	1530 bp	#461					
ORF	360	1889						
Allele	GB:HSSOX9MRN	460	866	866	A>G			
	source	isSNP	SNP00092616					
	consequence	GB:HSSOX9MRN.2	461	Silent	169-169	H		
Allele	GB:HSSOX9MRN	460	1571	1571	A>G			
	source	isSNP	SNP00108001					
	consequence	GB:HSSOX9MRN.2	461	Silent	404-404	P		
Allele	GB:HSSOX9MRN	460	1912	1912	G>T			
	source	isSNP	SNP00055269					
	consequence	GB:HSSOX9MRN.2	461	3'				
Allele	GB:HSSOX9MRN	460	2374	2374	A>G			
		255						

TABLE 1 (Cont.)

	source	isSNP	SNP00041454			
	consequence	GB:HSSOX9MRN.2	461	3'		
Allele	GB:HSSOX9MRN	460	3224	3224	C>G	
	source	isSNP	SNP00061027			
	consequence	GB:HSSOX9MRN.2	461	3'		
Allele	GB:HSSOX9MRN	460	3470	3470	A>G	
	source	isSNP	SNP00055270			
	consequence	GB:HSSOX9MRN.2	461	3'		
GIF SOX9-cdna-fwd.gif						
Link : FL_5425567_link_genomic						
Subsequence	GB:AC007461_8_5425567CD1		63884	60889	#462	
Subsequence	GB:AC007461_8	1	180385		#463	
Subsequence	SOX9_mrna_build.1	64243	58856		#464	
CDS	GB:AC007461_8_5425567CD1	1530 bp		3 exons		#462
exon	63884	63454				
exon	62557	62304				
exon	61733	60889				
mRNA	SOX9_mrna_build.1	3922 bp		3 exons		#464
exon	64243	63454				
exon	62557	62304				
exon	61733	58856				
Allele	GB:AC007461_8	463	59309	59309	A>G	
	source	isSNP	SNP00055270			
	consequence	GB:AC007461_8_5425567CD1		462	3'	
Allele	GB:AC007461_8	463	59555	59555	C>G	
	source	isSNP	SNP00061027			
	consequence	GB:AC007461_8_5425567CD1		462	3'	
Allele	GB:AC007461_8	463	60078	60078	A>G	
	source	isSNP	SNP00010889			
	consequence	GB:AC007461_8_5425567CD1		462	3'	
Allele	GB:AC007461_8	463	60404	60404	A>G	
	source	isSNP	SNP00041454			
	consequence	GB:AC007461_8_5425567CD1		462	3'	
Allele	GB:AC007461_8	463	60866	60866	G>T	
	source	isSNP	SNP00055269			
	consequence	GB:AC007461_8_5425567CD1		462	3'	
Allele	GB:AC007461_8	463	61207	61207	A>G	
	source	isSNP	SNP00108001			
	consequence	GB:AC007461_8_5425567CD1		462	Silent	
404-404	P					
Allele	GB:AC007461_8	463	62482	62482	A>G	
	source	isSNP	SNP00092616			
	source	wetSNP	GB:AC007461_8.v62482.G>A			
	consequence	GB:AC007461_8_5425567CD1		462	Silent	
169-169	H					
GIF SOX9-genomic-rev.gif						

## STATI2

Full name : STAT-induced STAT inhibitor-2

Link : FL\_2787140\_link\_cdna

Subsequence FN:2787140CB1 1 2587 #465

CDS FN:2787140CB1.1 927 bp #466

ORF 98 1024 256

TABLE 1 (Cont.)

Allele	FN:2787140CB1	465	1325	1325	A>G		
	source	isSNP	SNP00041483				
	consequence	FN:2787140CB1.1	466	3'			
Allele	FN:2787140CB1	465	1442	1442	G>T		
	source	isSNP	SNP00106962				
	consequence	FN:2787140CB1.1	466	3'			
Allele	FN:2787140CB1	465	1470	1470	A>G		
	source	isSNP	SNP00041484				
	consequence	FN:2787140CB1.1	466	3'			
Allele	FN:2787140CB1	465	1974	1974	A>G		
	source	isSNP	SNP00106963				
	consequence	FN:2787140CB1.1	466	3'			
GIF STATI2-cdna-fwd.gif							
Link : FL_1405668_link_genomic							
Subsequence	GB:AC012085_1	1	177866	#467			
Subsequence	FL_2787140_mrna_build.1	42013	47745	#468			
mRNA	FL_2787140_mrna_build.1	2580 bp	3 exons	#468			
exon	42013	42225					
exon	43694	44045					
exon	45731	47745					
Allele	GB:AC012085_1	467	44268	44268	A>G		
	source	isSNP	SNP00070304				
Allele	GB:AC012085_1	467	46492	46492	A>G		
	source	isSNP	SNP00041483				
Allele	GB:AC012085_1	467	46609	46609	G>T		
	source	isSNP	SNP00106962				
Allele	GB:AC012085_1	467	46637	46637	A>G		
	source	isSNP	SNP00041484				
Allele	GB:AC012085_1	467	47141	47141	A>G		
	source	isSNP	SNP00106963				
GIF STATI2-genomic-fwd.gif							
THBS1							
Full name : thrombospondin 1							
Link : THBS1_link_cdna							
Subsequence	GB:HSTS	1	5722	#469			
CDS	GB:HSTS.1	3513 bp	#470				
ORF	112	3624					
Allele	GB:HSTS	469	1239	1239	A>G		
	source	isSNP	SNP00046537				
	consequence	GB:HSTS.1	470	Silent	376-376	D	
Allele	GB:HSTS	469	2210	2210	A>G		
	source	isSNP	SNP00046539				
	consequence	GB:HSTS.1	470	Missense	700-700	N>S	
Allele	GB:HSTS	469	2979	2979	A>G		
	source	isSNP	SNP00061983				
	consequence	GB:HSTS.1	470	Silent	956-956	D	
Allele	GB:HSTS	469	3680	3680	G>T		
	source	isSNP	SNP00108514				
	consequence	GB:HSTS.1	470	3'			
Allele	GB:HSTS	469	3703	3703	A>G		
	source	isSNP	SNP00013197				
	consequence	GB:HSTS.1	470	3'			
			257				

TABLE 1 (Cont.)

Allele GB:HSTS 469 3905 3905 A>G  
 source isSNP SNP00093327  
 consequence GB:HSTS.1 470 3'  
 Allele GB:HSTS 469 5259 5259 A>G  
 source isSNP SNP00105437  
 consequence GB:HSTS.1 470 3'  
 GIF THBS1-cdna-fwd.gif

## TIMP1

Full name : Tissue Inhibitor of Metalloproteinase 1

Link : TIMP1\_link\_cdna

Subsequence FN:411388CB1 1 853 #471  
 CDS FN:411388CB1.1 621 bp #472  
 ORF 122 742  
 Allele FN:411388CB1 471 365 365 C>G  
 source isSNP SNP00115174  
 consequence FN:411388CB1.1 472 Missense 82-82 R>G  
 GIF TIMP1-cdna-fwd.gif

Link : FL\_3013907\_link\_genomic

Subsequence GB:HS230G1 1 125515 #473  
 Subsequence GB:HS230G1\_411388CD1 20559 17287 #474  
 Subsequence TIMP1\_mrna\_build.1 21613 17186 #475  
 mRNA TIMP1\_mrna\_build.1 843 bp 6 exons #475  
 exon 21613 21501  
 exon 20567 20439  
 exon 19039 18960  
 exon 18770 18644  
 exon 18432 18308  
 exon 17454 17186  
 CDS GB:HS230G1\_411388CD1 621 bp 5 exons #474  
 exon 20559 20439  
 exon 19039 18960  
 exon 18770 18644  
 exon 18432 18308  
 exon 17454 17287  
 Allele GB:HS230G1 473 17434 17434 A>G  
 source wetSNP GB:HS230G1.v17434.G>A  
 consequence GB:HS230G1\_411388CD1 474 Silent 158-158  
 I  
 Allele GB:HS230G1 473 17550 17550 A>G  
 source isSNP SNP00099224  
 consequence GB:HS230G1\_411388CD1 474 Intron  
 Allele GB:HS230G1 473 18046 18046 A>G  
 source isSNP SNP00099223  
 consequence GB:HS230G1\_411388CD1 474 Intron  
 Allele GB:HS230G1 473 18088 18088 A>G  
 source isSNP SNP00030937  
 consequence GB:HS230G1\_411388CD1 474 Intron  
 Allele GB:HS230G1 473 18389 18389 A>G  
 source wetSNP GB:HS230G1.v18389.A>G  
 consequence GB:HS230G1\_411388CD1 474 Silent 124-124  
 F  
 Allele GB:HS230G1 473 18495 18495 C>G  
 258



TABLE 1 (Cont.)

	source	isSNP	SNP00099222			
	source	wetSNP	GB:HS230G1.v18495.C>G			
	consequence	GB:HS230G1_411388CD1	474	Intron		
Allele	GB:HS230G1	473	18711	18711	A>G	
	source	wetSNP	GB:HS230G1.v18711.G>A			
	consequence	GB:HS230G1_411388CD1	474	Silent		87-87 P
Allele	GB:HS230G1	473	18728	18728	C>G	
	source	isSNP	SNP00115174			
	consequence	GB:HS230G1_411388CD1	474	Missense		82-82 R>G

GIF TIMP1-genomic-rev.gif

## TIMP2

Full name : Tissue Inhibitor of Metalloproteinase-2.

Link : TIMP2\_link\_genomic

Subsequence	TIMP2_cds.1	822	3126	#476		
Subsequence	GB:S68860_1	1	970	#477		
Subsequence	GB:U44382_1	1071	1320	#478		
Subsequence	GB:U44383_1	1421	1644	#479		
Subsequence	GB:U44384_1	1745	2283	#480		
Subsequence	GB:U44385_1	2384	3750	#481		
Subsequence	TIMP2_mrna_build.1		810	3251	#482	
CDS	TIMP2_cds.1	663 bp	5 exons	#476		
exon	822	951				
exon	1125	1225				
exon	1504	1612				
exon	1939	2063				
exon	2929	3126				
mRNA	TIMP2_mrna_build.1		800 bp	5 exons	#482	
exon	810	951				
exon	1125	1225				
exon	1504	1612				
exon	1939	2063				
exon	2929	3251				
Allele	GB:U44383_1	479	155	155	A>G	
	source	wetSNP	GB:U44383_1.v155.G>A			
	consequence	TIMP2_cds.1	476	Silent		101-101 S

GIF TIMP2-genomic-fwd.gif

## TNA

Full name : tetranectin

Link : TNA\_link\_cdna

Subsequence	GB:NM_003278	1	874	#483		
CDS	GB:NM_003278.1	609 bp		#484		
ORF	94	702				
Allele	GB:NM_003278	483	409	409	A>G	
	source	isSNP	SNP00007942			
	consequence	GB:NM_003278.1	484	Missense		106-106 S>G
Allele	GB:NM_003278	483	744	744	A>G	
	source	isSNP	SNP00007943			
	consequence	GB:NM_003278.1	484	3'		

GIF TNA-cdna-fwd.gif

TABLE 1 (Cont.)

Link : TNA\_link\_genomic

Subsequence	TNA_cds.1	254	1629	#485		
Subsequence	TNA_cds.2	254	1629	#486		
Subsequence	GB:X70910_1	1	570	#487		
Subsequence	GB:X70911_1	671	978	#488		
Subsequence	GB:X70912_1	1079	1805	#489		
Subsequence	TNA_mrna_build.1	164	1776	#490		
CDS	TNA_cds.1	609 bp	3 exons	#485		
	exon	254	362			
	exon	829	927			
	exon	1229	1629			
CDS	TNA_cds.2	510 bp	2 exons	#486		
	exon	254	362			
	exon	1229	1629			
mRNA	TNA_mrna_build.1	846 bp	3 exons	#490		
	exon	164	362			
	exon	829	927			
	exon	1229	1776			
Allele	GB:X70912_1	489	258	258	A>G	
	source	isSNP	SNP00007942			
	consequence	TNA_cds.1	485	Missense	106-106	S>G
	consequence	TNA_cds.2	486	Missense	73-73	S>G
Allele	GB:X70912_1	489	593	593	A>G	
	source	isSNP	SNP00007943			
	consequence	TNA_cds.1	485	3'		
	consequence	TNA_cds.2	486	3'		
GIF	TNA-genomic-fwd.gif					

## TNFAIP6

Full name : tumor necrosis factor, alpha-induced protein 6

Link : TNFAIP6\_link\_cdna

Subsequence	GB:NM_007115_1	1	1414	#491		
CDS	GB:NM_007115_1.1	834 bp		#492		
	ORF	69	902			
Allele	GB:NM_007115_1	491	499	499	A>G	
	source	isSNP	SNP00040822			
	consequence	GB:NM_007115_1.1	492	Missense	144-144	R>Q
Allele	GB:NM_007115_1	491	1143	1143	C>G	
	source	isSNP	SNP00040823			
	consequence	GB:NM_007115_1.1	492	3'		

GIF TNFAIP6-cdna-fwd.gif

Link : FL\_1000909\_link\_genomic

Subsequence	GB:AC009311_1_191918CD1	132384	154250	#493		
Subsequence	GB:AC009311_1	1	160198	#494		
Subsequence	TNFAIP6_mrna_build.1	132314	154760	#495		
mRNA	TNFAIP6_mrna_build.1	1414 bp	6 exons	#495		
	exon	132314	132477			
	exon	138660	138797			
	exon	140773	140934			
	exon	144737	144965			
	exon	148266	148306			
	exon	154081	154760			
CDS	GB:AC009311_1_191918CD1	834 bp	260	6 exons	#493	

TABLE 1 (Cont.)

exon	132384	132477				
exon	138660	138797				
exon	140773	140934				
exon	144737	144965				
exon	148266	148306				
exon	154081	154250				
Allele	GB:AC009311_1	494	140934	140934	A>G	
	source	wetSNP	GB:AC009311_1.v140934.G>A			
	consequence	GB:AC009311_1_191918CD1	493	Missense		132-132
A>T						
Allele	GB:AC009311_1	494	140942	140942	A>T	
	source	wetSNP	GB:AC009311_1.v140942.A>T			
	consequence	GB:AC009311_1_191918CD1	493	Intron		
Allele	GB:AC009311_1	494	144773	144773	A>G	
	source	isSNP	SNP00040822			
	source	wetSNP	GB:AC009311_1.v144773.A>G			
	consequence	GB:AC009311_1_191918CD1	493	Missense		144-144
Q>R						
Allele	GB:AC009311_1	494	148030	148030	A>G	
	source	dbSNP	gnl dbSNP ss645109_allele			
	consequence	GB:AC009311_1_191918CD1	493	Intron		
Allele	GB:AC009311_1	494	148229	148229	A>G	
	source	wetSNP	GB:AC009311_1.v148229.T>C			
	consequence	GB:AC009311_1_191918CD1	493	Intron		
Allele	GB:AC009311_1	494	148245	148245	A>G	
	source	wetSNP	GB:AC009311_1.v148245.T>C			
	consequence	GB:AC009311_1_191918CD1	493	Intron		
Allele	GB:AC009311_1	494	154493	154493	C>G	
	source	isSNP	SNP00040823			
	consequence	GB:AC009311_1_191918CD1	493	3'		

GIF TNFAIP6-genomic-fwd.gif

## TNFRSF11B

Full name : TNFRSF11B

Link : TNFRSF11B\_link\_cdna

Subsequence GB:AB002146 1 1206 #496

CDS GB:AB002146.1 1206 bp #497

ORF 1 1206

Allele GB:AB002146 496 768 768 A&gt;G

source isSNP SNP00028816

consequence GB:AB002146.1 497 Silent 256-256 L

GIF TNFRSF11B-cdna-fwd.gif

Link : TNFRSF11B\_link\_genomic

Subsequence TNFRSF11B\_cds.1 125 9057 #498

Subsequence GB:E15270\_1 1 9898 #499

CDS TNFRSF11B\_cds.1 1176 bp 4 exons #498

exon 130 499

exon 4504 4695

exon 6716 6940

exon 8669 9057

Allele GB:E15270\_1 499 503 503 A&gt;G

source wetSNP GB:E15270\_1.v503.C&gt;T

consequence TNFRSF11B\_cds.1 498 Intron

TABLE 1 (Cont.)

Allele	GB:E15270_1	499	4499	4499	A>G			
	source	wetSNP			GB:E15270_1.v4499.C>T			
	consequence	TNFRSF11B_cds.1	498	Intron				
Allele	GB:E15270_1	499	4661	4661	A>G			
	source	wetSNP			GB:E15270_1.v4661.C>T			
	consequence	TNFRSF11B_cds.1	498	Silent		176-176		S
Allele	GB:E15270_1	499	4749	4752	TCTG>TG			
	source	wetSNP			GB:E15270_1.v4749.TCTG>TG			
	consequence	TNFRSF11B_cds.1	498	Intron				
Allele	GB:E15270_1	499	6599	6599	A>G			
	source	wetSNP			GB:E15270_1.v6599.G>A			
	consequence	TNFRSF11B_cds.1	498	Intron				
Allele	GB:E15270_1	499	6837	6837	A>G			
	source	wetSNP			GB:E15270_1.v6837.G>A			
	consequence	TNFRSF11B_cds.1	498	Silent		228-228		E
Allele	GB:E15270_1	499	6891	6891	A>G			
	source	isSNP	SNP00028816					
	consequence	TNFRSF11B_cds.1	498	Silent		246-246		L
GIF TNFRSF11B-genomic-fwd.gif								

TABLE 2

Gene	Sequence	Seq Offset	Pol	DNA change	Qualifer	Peptide change	Codon	No. ind. screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
AACT	GB:AL049839_2	59566	148	G > A	MIS	A > T	9	47	0.5	14	19	14	all
AACT	GB:AL049839_2	59566	148	G > A	MIS	A > T	9	10	0.2	7	2	1	AFA
AACT	GB:AL049839_2	59566	148	G > A	MIS	A > T	9	13	0.7308	0	7	6	ASI
AACT	GB:AL049839_2	59566	148	G > A	MIS	A > T	9	17	0.6471	1	10	6	CAU
AACT	GB:AL049839_2	59566	148	G > A	MIS	A > T	9	7	0.1429	6	0	1	HIS
AACT	GB:AL049839_2	68882	55	A > G	MIS	M > V	414	47	0.0106	46	1	0	all
AACT	GB:AL049839_2	68882	55	A > G	MIS	M > V	414	10	0	10	0	0	AFA
AACT	GB:AL049839_2	68882	55	A > G	MIS	M > V	414	13	0.0385	12	1	0	ASI
AACT	GB:AL049839_2	68882	55	A > G	MIS	M > V	414	17	0	17	0	0	CAU
AACT	GB:AL049839_2	68882	55	A > G	MIS	M > V	414	7	0	7	0	0	HIS
ABL1	GB:U07563_1	58876	85	C > G	NCD			47	0.0106	46	1	0	all
ABL1	GB:U07563_1	58876	85	C > G	NCD			10	0	10	0	0	AFA
ABL1	GB:U07563_1	58876	85	C > G	NCD			13	0.0385	12	1	0	ASI
ABL1	GB:U07563_1	58876	85	C > G	NCD			17	0	17	0	0	CAU
ABL1	GB:U07563_1	58876	85	C > G	NCD			7	0	7	0	0	HIS
ABL1	GB:U07563_1	58876	121	T > C	NCD			47	0.0319	44	3	0	all
ABL1	GB:U07563_1	68640	121	T > C	NCD			10	0.15	7	3	0	AFA
ABL1	GB:U07563_1	68640	121	T > C	NCD			13	0	13	0	0	ASI
ABL1	GB:U07563_1	68640	121	T > C	NCD			17	0	17	0	0	CAU
ABL1	GB:U07563_1	68640	121	T > C	NCD			7	0	7	0	0	HIS
ABL1	GB:U07563_1	68640	121	T > C	NCD			47	0.0745	40	7	0	all
ABL1	GB:U07563_1	74901	143	A > G	SIL	E	499	47	0.1	8	2	0	AFA
ABL1	GB:U07563_1	74901	143	A > G	SIL	E	499	10	0.1	13	0	0	ASI
ABL1	GB:U07563_1	74901	143	A > G	SIL	E	499	13	0	13	0	0	CAU
ABL1	GB:U07563_1	74901	143	A > G	SIL	E	499	17	0.1176	13	4	0	HIS
ABL1	GB:U07563_1	74901	143	A > G	SIL	E	499	7	0.0714	6	1	0	all
ABL1	GB:U07563_1	78921	87	G > A	SIL	E	623	46	0.0435	42	4	0	AFA
ABL1	GB:U07563_1	78921	87	G > A	SIL	E	623	10	0.2	6	4	0	ASI
ABL1	GB:U07563_1	78921	87	G > A	SIL	E	623	13	0	13	0	0	CAU
ABL1	GB:U07563_1	78921	87	G > A	SIL	E	623	17	0	17	0	0	HIS
ABL1	GB:U07563_1	78921	87	G > A	SIL	E	623	6	0	6	0	0	all
ABL1	GB:U07563_1	78921	87	G > A	SIL	E	623	47	0.0106	46	1	0	AFA
ABL1	GB:U07563_1	79239	136	G > A	SIL	T	729	10	0.05	9	1	0	ASI
ABL1	GB:U07563_1	79239	136	G > A	SIL	T	729	13	0	13	0	0	CAU
ABL1	GB:U07563_1	79239	136	G > A	SIL	T	729	17	0	17	0	0	HIS
ABL1	GB:U07563_1	79239	136	G > A	SIL	T	729	7	0	7	0	0	all
ABL1	GB:U07563_1	79239	136	G > A	SIL	T	729	46	0.0761	39	7	0	AFA
ABL1	GB:U07563_1	79404	125	C > G	SIL	P	784	10	0.15	7	3	0	ASI
ABL1	GB:U07563_1	79404	125	C > G	SIL	P	784	13	0	13	0	0	CAU
ABL1	GB:U07563_1	79404	125	C > G	SIL	P	784	16	0.0938	13	3	0	HIS
ABL1	GB:U07563_1	79404	125	C > G	SIL	P	784	7	0.0714	6	1	0	all

TABLE 2

Gene	Sequence	Seq Offset	Pol Position	DNA change	Qualifier	Peptide change	Codon	No. ind. screened	Freq. of variant	No. AA	No. AB	No. BB	Ethnic group
ABL1	GB:U07563_1	79657	171	C > T	MIS	P > S	869	47	0.0106	46	1	0	all
ABL1	GB:U07563_1	79657	171	C > T	MIS	P > S	869	10	0	10	0	0	AFA
ABL1	GB:U07563_1	79657	171	C > T	MIS	P > S	869	13	0	13	0	0	ASI
ABL1	GB:U07563_1	79657	171	C > T	MIS	P > S	869	17	0.0294	16	1	0	CAU
ABL1	GB:U07563_1	79657	171	C > T	MIS	P > S	869	7	0	7	0	0	HIS
ABL1	GB:U07563_1	79750	76	C > T	MIS	P > S	900	46	0.0109	45	1	0	all
ABL1	GB:U07563_1	79750	76	C > T	MIS	P > S	900	10	0.05	9	1	0	AFA
ABL1	GB:U07563_1	79750	76	C > T	MIS	P > S	900	13	0	13	0	0	ASI
ABL1	GB:U07563_1	79750	76	C > T	MIS	P > S	900	16	0	16	0	0	CAU
ABL1	GB:U07563_1	80376	70	C > T	MIS	P > S	900	7	0	7	0	0	HIS
ABL1	GB:U07563_1	80376	70	G > A	SIL	P	1108	47	0.4894	1	46	0	all
ABL1	GB:U07563_1	80376	70	G > A	SIL	P	1108	10	0.5	0	10	0	AFA
ABL1	GB:U07563_1	80376	70	G > A	SIL	P	1108	13	0.5	0	13	0	ASI
ABL1	GB:U07563_1	80376	70	G > A	SIL	P	1108	17	0.4706	1	16	0	CAU
ABL1	GB:U07563_1	80376	70	G > A	SIL	P	1108	7	0.5	0	7	0	HIS
ACLP	GB:AC006454_3	141107	197	GA > GGA	NCD			44	0.0114	43	1	0	all
ACLP	GB:AC006454_3	141107	197	GA > GGA	NCD			9	0	9	0	0	AFA
ACLP	GB:AC006454_3	141107	197	GA > GGA	NCD			12	0	12	0	0	ASI
ACLP	GB:AC006454_3	141107	197	GA > GGA	NCD			17	0.0294	16	1	0	CAU
ACLP	GB:AC006454_3	141107	197	GA > GGA	NCD			6	0	6	0	0	HIS
ACLP	GB:AC006454_3	142383	132	T > C	MIS	T > A	424	47	0.0106	46	1	0	all
ACLP	GB:AC006454_3	142383	132	T > C	MIS	T > A	424	10	0	10	0	0	AFA
ACLP	GB:AC006454_3	142383	132	T > C	MIS	T > A	424	13	0	13	0	0	ASI
ACLP	GB:AC006454_3	142383	132	T > C	MIS	T > A	424	17	0.0294	16	1	0	CAU
ACLP	GB:AC006454_3	142383	132	T > C	MIS	T > A	424	7	0	7	0	0	HIS
ACLP	GB:AC006454_3	145005	21	C > T	NCD			46	0.0217	44	2	0	all
ACLP	GB:AC006454_3	145005	21	C > T	NCD			10	0	10	0	0	AFA
ACLP	GB:AC006454_3	145005	21	C > T	NCD			12	0	12	0	0	ASI
ACLP	GB:AC006454_3	145005	21	C > T	NCD			17	0.0588	15	2	0	CAU
ACLP	GB:AC006454_3	145005	21	C > T	NCD			7	0	7	0	0	HIS
ANK	GB:AC026437_2	25779	182	C > T	SIL	A	51	47	0.117	37	9	1	all
ANK	GB:AC026437_2	25779	182	C > T	SIL	A	51	10	0.15	7	3	0	AFA
ANK	GB:AC026437_2	25779	182	C > T	SIL	A	51	13	0	13	0	0	ASI
ANK	GB:AC026437_2	25779	182	C > T	SIL	A	51	17	0.2059	11	5	1	CAU
ANK	GB:AC026437_2	25779	182	C > T	SIL	A	51	7	0.0714	6	1	0	HIS
ANK	GB:AC026437_2	25807	210	G > A	NCD			47	0.0745	41	5	1	all
ANK	GB:AC026437_2	25807	210	G > A	NCD			10	0	10	0	0	AFA
ANK	GB:AC026437_2	25807	210	G > A	NCD			13	0	13	0	0	ASI
ANK	GB:AC026437_2	25807	210	G > A	NCD			17	0.1765	12	4	1	CAU
ANK	GB:AC026437_2	25807	210	G > A	NCD			7	0.0714	6	1	0	HIS

TABLE 2

Gene	Sequence	Seq Offset	Position	Pol	DNA change	Qualifier	Peptide change	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
ANK	GB:AC026437_2	36172	24		T > C	NCD			46	0.0109	45	1	0	all
ANK	GB:AC026437_2	36172	24		T > C	NCD			10	0	10	0	0	AFA
ANK	GB:AC026437_2	36172	24		T > C	NCD			13	0.0385	12	1	0	ASI
ANK	GB:AC026437_2	36172	24		T > C	NCD			17	0	17	0	0	CAU
ANK	GB:AC026437_2	36172	24		T > C	NCD			6	0	6	0	0	HIS
ANK	GB:AC026437_2	52817	21		C > A	NCD			47	0.0106	46	1	0	all
ANK	GB:AC026437_2	52817	21		C > A	NCD			10	0	10	0	0	AFA
ANK	GB:AC026437_2	52817	21		C > A	NCD			13	0.0385	12	1	0	ASI
ANK	GB:AC026437_2	52817	21		C > A	NCD			17	0	17	0	0	CAU
ANK	GB:AC026437_2	52817	21		C > A	NCD			7	0	7	0	0	HIS
ANK	GB:AC026437_2	52899	103		A > G	SIL	A	274	47	0.0851	40	6	1	all
ANK	GB:AC026437_2	52899	103		A > G	SIL	A	274	10	0.05	9	1	0	AFA
ANK	GB:AC026437_2	52899	103		A > G	SIL	A	274	13	0.1538	10	2	1	ASI
ANK	GB:AC026437_2	52899	103		A > G	SIL	A	274	17	0.0294	16	1	0	CAU
ANK	GB:AC026437_2	52899	103		A > G	SIL	A	274	7	0.1429	5	2	0	HIS
ANK	GB:AC026437_2	52899	40		A > G	SIL	A	274	47	0.0851	40	6	1	all
ANK	GB:AC026437_2	52899	40		A > G	SIL	A	274	10	0.05	9	1	0	AFA
ANK	GB:AC026437_2	52899	40		A > G	SIL	A	274	13	0.1538	10	2	1	ASI
ANK	GB:AC026437_2	52899	40		A > G	SIL	A	274	17	0.0294	16	1	0	CAU
ANK	GB:AC026437_2	52899	40		A > G	SIL	A	274	7	0.1429	5	2	0	HIS
ANK	GB:AC026437_2	52899	40		A > G	SIL	A	274	47	0.0745	40	7	0	all
ANK	GB:AC026437_2	52962	103		T > G	NCD			10	0.05	9	1	0	AFA
ANK	GB:AC026437_2	52962	103		T > G	NCD			13	0	13	0	0	ASI
ANK	GB:AC026437_2	52962	103		T > G	NCD			17	0.1176	13	4	0	CAU
ANK	GB:AC026437_2	52962	103		T > G	NCD			7	0.1429	5	2	0	HIS
ANK	GB:AC026437_2	81235	144		T > C	NCD			47	0.0106	46	1	0	all
ANK	GB:AC026437_2	81235	144		T > C	NCD			10	0.05	9	1	0	AFA
ANK	GB:AC026437_2	81235	144		T > C	NCD			13	0	13	0	0	ASI
ANK	GB:AC026437_2	81235	144		T > C	NCD			17	0	17	0	0	CAU
ANK	GB:AC026437_2	81235	144		T > C	NCD			7	0	7	0	0	HIS
ANK	GB:AC026437_2	83587	186		G > A	NCD			47	0.0106	46	1	0	all
ANK	GB:AC026437_2	83587	186		G > A	NCD			10	0	10	0	0	AFA
ANK	GB:AC026437_2	83587	186		G > A	NCD			13	0.0385	12	1	0	ASI
ANK	GB:AC026437_2	83587	186		G > A	NCD			17	0	17	0	0	CAU
ANK	GB:AC026437_2	83587	186		G > A	NCD			7	0	7	0	0	HIS
ANK	GB:AC026437_2	83607	206		A > G	NCD			47	0.0745	40	7	0	all
ANK	GB:AC026437_2	83607	206		A > G	NCD			10	0.2	6	4	0	AFA
ANK	GB:AC026437_2	83607	206		A > G	NCD			13	0	13	0	0	ASI
ANK	GB:AC026437_2	83607	206		A > G	NCD			17	0.0588	15	2	0	CAU
ANK	GB:AC026437_2	83607	206		A > G	NCD			7	0.0714	6	1	0	HIS

TABLE 2

Gene	Sequence	Seq Offset	Pol Position	DNA change	Peptide change	Qualifier	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
BGLAP	GB:AC007227_2	34618	75	G > C	A	SIL	92	47	0.0319	44	3	0	all
BGLAP	GB:AC007227_2	34618	75	G > C	A	SIL	92	10	0.15	7	3	0	AFA
BGLAP	GB:AC007227_2	34618	75	G > C	A	SIL	92	13	0	13	0	0	ASI
BGLAP	GB:AC007227_2	34618	75	G > C	A	SIL	92	17	0	17	0	0	CAU
BGLAP	GB:AC007227_2	34618	75	G > C	A	SIL	92	7	0	7	0	0	HIS
BGLAP	GB:AC007227_2	34977	79	G > T	Q > K	MIS	40	47	0.0319	44	3	0	all
BGLAP	GB:AC007227_2	34977	79	G > T	Q > K	MIS	40	10	0	10	0	0	AFA
BGLAP	GB:AC007227_2	34977	79	G > T	Q > K	MIS	40	13	0.1154	10	3	0	ASI
BGLAP	GB:AC007227_2	34977	79	G > T	Q > K	MIS	40	17	0	17	0	0	CAU
BGLAP	GB:AC007227_2	34977	79	G > T	Q > K	MIS	40	7	0	7	0	0	HIS
BGN	GB:U82695	18161	56	A > G	E	SIL	40	47	0.0106	46	1	0	all
BGN	GB:U82695	18161	56	A > G	E	SIL	40	10	0.05	9	1	0	AFA
BGN	GB:U82695	18161	56	A > G	E	SIL	40	13	0	13	0	0	ASI
BGN	GB:U82695	18161	56	A > G	E	SIL	40	17	0	17	0	0	CAU
BGN	GB:U82695	18161	56	A > G	E	SIL	40	7	0	7	0	0	HIS
BGN	GB:U82695	18182	77	G > A	S	SIL	47	47	0.4681	17	16	14	all
BGN	GB:U82695	18182	77	G > A	S	SIL	47	10	0.4	4	4	2	AFA
BGN	GB:U82695	18182	77	G > A	S	SIL	47	13	0.5385	5	2	6	ASI
BGN	GB:U82695	18182	77	G > A	S	SIL	47	17	0.4706	5	8	4	CAU
BGN	GB:U82695	18182	77	G > A	S	SIL	47	7	0.4286	3	2	2	HIS
BGN	GB:U82695	18330	225	G > A	NCD	NCD		47	0.0319	45	1	1	all
BGN	GB:U82695	18330	225	G > A	NCD	NCD		10	0.05	9	1	0	AFA
BGN	GB:U82695	18330	225	G > A	NCD	NCD		13	0	13	0	0	ASI
BGN	GB:U82695	18330	225	G > A	NCD	NCD		17	0.0588	16	0	1	CAU
BGN	GB:U82695	18330	225	G > A	NCD	NCD		7	0	7	0	0	HIS
BGN	GB:U82695	18354	249	G > A	NCD	NCD		47	0.1064	42	0	5	all
BGN	GB:U82695	18354	249	G > A	NCD	NCD		10	0.2	8	0	2	AFA
BGN	GB:U82695	18354	249	G > A	NCD	NCD		13	0.0769	12	0	1	ASI
BGN	GB:U82695	18354	249	G > A	NCD	NCD		17	0.0588	16	0	1	CAU
BGN	GB:U82695	18354	249	G > A	NCD	NCD		7	0.1429	6	0	1	HIS
BGN	GB:U82695	19460	230	T > C	S	SIL	180	46	0.6957	9	10	27	all
BGN	GB:U82695	19460	230	T > C	S	SIL	180	9	0.8333	1	1	7	AFA
BGN	GB:U82695	19460	230	T > C	S	SIL	180	13	0.6923	3	2	8	ASI
BGN	GB:U82695	19460	230	T > C	S	SIL	180	17	0.6765	3	5	9	CAU
BGN	GB:U82695	19460	230	T > C	S	SIL	180	7	0.5714	2	2	3	HIS
BGN	GB:U82695	21566	22	G > T	NCD	NCD		47	0.1383	36	9	2	all
BGN	GB:U82695	21566	22	G > T	NCD	NCD		10	0.05	9	1	0	AFA
BGN	GB:U82695	21566	22	G > T	NCD	NCD		13	0.0769	12	0	1	ASI
BGN	GB:U82695	21566	22	G > T	NCD	NCD		17	0.2353	10	6	1	CAU
BGN	GB:U82695	21566	22	G > T	NCD	NCD		7	0.1429	5	2	0	HIS



TABLE 2

Gene	Sequence	Seq Offset	Pol Position	DNA change	Qualifier	Peptide change	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
BGN	GB:U82695	21639	95	C > T	NCD			47	0.0426	44	2	1	all
BGN	GB:U82695	21639	95	C > T	NCD			10	0	10	0	0	AFA
BGN	GB:U82695	21639	95	C > T	NCD			13	0.1538	10	2	1	ASI
BGN	GB:U82695	21639	95	C > T	NCD			17	0	17	0	0	CAU
BGN	GB:U82695	21639	95	C > T	NCD			7	0	7	0	0	HIS
BMP2	GB:HS859D4	168341	121	T > A	MIS	R>S	190	47	0.766	4	14	29	all
BMP2	GB:HS859D4	168341	121	T > A	MIS	R>S	190	10	0.8	0	4	6	AFA
BMP2	GB:HS859D4	168341	121	T > A	MIS	R>S	190	13	0.7692	2	2	9	ASI
BMP2	GB:HS859D4	168341	121	T > A	MIS	R>S	190	17	0.7059	2	6	9	CAU
BMP2	GB:HS859D4	168341	121	T > A	MIS	R>S	190	7	0.8571	0	2	5	HIS
BMP4	GB:HSU43842	9215	189	C > T	MIS	A>V	152	43	0.3721	27	0	16	all
BMP4	GB:HSU43842	9215	189	C > T	MIS	A>V	152	8	0.5	4	0	4	AFA
BMP4	GB:HSU43842	9215	189	C > T	MIS	A>V	152	11	0.5455	5	0	6	ASI
BMP4	GB:HSU43842	9215	189	C > T	MIS	A>V	152	17	0.2353	13	0	4	CAU
BMP4	GB:HSU43842	9215	189	C > T	MIS	A>V	152	7	0.2857	5	0	2	HIS
BMP4	GB:HSU43842	9215	30	C > T	MIS	A>V	152	47	0.766	11	0	36	all
BMP4	GB:HSU43842	9215	30	C > T	MIS	A>V	152	10	0.9	1	0	9	AFA
BMP4	GB:HSU43842	9215	30	C > T	MIS	A>V	152	13	0.8462	2	0	11	ASI
BMP4	GB:HSU43842	9215	30	C > T	MIS	A>V	152	17	0.7059	5	0	12	CAU
BMP4	GB:HSU43842	9215	30	C > T	MIS	A>V	152	7	0.5714	3	0	4	HIS
CBFA1	GB:HSCBFA1S3	177	88	C > T	SIL	N	183	46	0.0109	45	1	0	all
CBFA1	GB:HSCBFA1S3	177	88	C > T	SIL	N	183	10	0.05	9	1	0	AFA
CBFA1	GB:HSCBFA1S3	177	88	C > T	SIL	N	183	13	0	13	0	0	ASI
CBFA1	GB:HSCBFA1S3	177	88	C > T	SIL	N	183	17	0	17	0	0	CAU
CBFA1	GB:HSCBFA1S3	177	88	C > T	SIL	N	183	6	0	6	0	0	HIS
CBFA1	GB:HSCBFA1S8	490	42	C > T	SIL	S	503	46	0.0109	45	1	0	all
CBFA1	GB:HSCBFA1S8	490	42	C > T	SIL	S	503	9	0	9	0	0	AFA
CBFA1	GB:HSCBFA1S8	490	42	C > T	SIL	S	503	13	0.0385	12	1	0	ASI
CBFA1	GB:HSCBFA1S8	490	42	C > T	SIL	S	503	17	0	17	0	0	CAU
CBFA1	GB:HSCBFA1S8	490	42	C > T	SIL	S	503	7	0	7	0	0	HIS
CD36	EM:HSCD36G10	92	74	T > C	SIL	F	293	46	0.0109	45	1	0	all
CD36	EM:HSCD36G10	92	74	T > C	SIL	F	293	10	0	10	0	0	AFA
CD36	EM:HSCD36G10	92	74	T > C	SIL	F	293	13	0.0385	12	1	0	ASI
CD36	EM:HSCD36G10	92	74	T > C	SIL	F	293	16	0	16	0	0	CAU
CD36	EM:HSCD36G10	92	74	T > C	SIL	F	293	7	0	7	0	0	HIS
CD68	GB:AC007421_12	90707	123	C > T	MIS	A>T	340	47	0.0106	46	1	0	all
CD68	GB:AC007421_12	90707	123	C > T	MIS	A>T	340	10	0	10	0	0	AFA
CD68	GB:AC007421_12	90707	123	C > T	MIS	A>T	340	13	0	13	0	0	ASI
CD68	GB:AC007421_12	90707	123	C > T	MIS	A>T	340	17	0.0294	16	1	0	CAU
CD68	GB:AC007421_12	90707	123	C > T	MIS	A>T	340	7	0	7	0	0	HIS

TABLE 2

Gene	Sequence	Seq Offset	Position	Pol	DNA change	Qualifier	Peptide change	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
CD68	GB:AC007421_12	91388	62		G > T	SPL			47	0.1064	37	10	0	all
CD68	GB:AC007421_12	91388	62		G > T	SPL			10	0.05	9	1	0	AFA
CD68	GB:AC007421_12	91388	62		G > T	SPL			13	0.1538	9	4	0	ASI
CD68	GB:AC007421_12	91388	62		G > T	SPL			17	0.1176	13	4	0	CAU
CD68	GB:AC007421_12	91388	62		G > T	SPL			7	0.0714	6	1	0	HIS
CILP	GB:AB022430_1	3567	39		A > C	NCD			47	0.0106	46	1	0	all
CILP	GB:AB022430_1	3567	39		A > C	NCD			10	0.05	9	1	0	AFA
CILP	GB:AB022430_1	3567	39		A > C	NCD			13	0	13	0	0	ASI
CILP	GB:AB022430_1	3567	39		A > C	NCD			17	0	17	0	0	CAU
CILP	GB:AB022430_1	3567	39		A > C	NCD			7	0	7	0	0	HIS
CILP	GB:AB022430_1	11286	61		T > A	NCD			47	0.0106	46	1	0	all
CILP	GB:AB022430_1	11286	61		T > A	NCD			10	0.05	9	1	0	AFA
CILP	GB:AB022430_1	11286	61		T > A	NCD			13	0	13	0	0	ASI
CILP	GB:AB022430_1	11286	61		T > A	NCD			17	0	17	0	0	CAU
CILP	GB:AB022430_1	11286	61		T > A	NCD			7	0	7	0	0	HIS
CILP	GB:AB022430_1	11286	61		T > A	NCD			47	0.5106	16	14	17	all
CILP	GB:AB022430_1	11491	71		C > T	MIS	T>I	395	47	0.25	5	5	0	AFA
CILP	GB:AB022430_1	11491	71		C > T	MIS	T>I	395	10	0.8462	1	2	10	ASI
CILP	GB:AB022430_1	11491	71		C > T	MIS	T>I	395	13	0.5	6	5	6	CAU
CILP	GB:AB022430_1	11491	71		C > T	MIS	T>I	395	17	0.2857	4	2	1	HIS
CILP	GB:AB022430_1	11491	71		C > T	MIS	T>I	395	7	0.0106	46	1	0	all
CILP	GB:AB022430_1	14421	136		G > C	MIS	R>T	446	47	0	10	0	0	AFA
CILP	GB:AB022430_1	14421	136		G > C	MIS	R>T	446	10	0	12	1	0	ASI
CILP	GB:AB022430_1	14421	136		G > C	MIS	R>T	446	13	0.0385	17	0	0	CAU
CILP	GB:AB022430_1	14421	136		G > C	MIS	R>T	446	17	0	17	0	0	HIS
CILP	GB:AB022430_1	14421	136		G > C	MIS	R>T	446	7	0	7	0	0	all
CILP	GB:AB022430_1	15116	122		G > A	MIS	V>M	678	47	0.0106	46	1	0	AFA
CILP	GB:AB022430_1	15116	122		G > A	MIS	V>M	678	10	0	10	0	0	ASI
CILP	GB:AB022430_1	15116	122		G > A	MIS	V>M	678	13	0.0385	12	1	0	CAU
CILP	GB:AB022430_1	15116	122		G > A	MIS	V>M	678	17	0	17	0	0	HIS
CILP	GB:AB022430_1	15116	122		G > A	MIS	V>M	678	7	0	7	0	0	all
CILP	GB:AB022430_1	15670	146		G > A	SIL	T	862	47	0.0106	46	1	0	AFA
CILP	GB:AB022430_1	15670	146		G > A	SIL	T	862	10	0.05	9	1	0	ASI
CILP	GB:AB022430_1	15670	146		G > A	SIL	T	862	13	0	13	0	0	CAU
CILP	GB:AB022430_1	15670	146		G > A	SIL	T	862	17	0	17	0	0	HIS
CILP	GB:AB022430_1	15670	146		G > A	SIL	T	862	7	0	7	0	0	all
CTSC	GB:AC011088_8	124932	57		A > T	NCD			47	0.0106	46	1	0	AFA
CTSC	GB:AC011088_8	124932	57		A > T	NCD			10	0.05	9	1	0	ASI
CTSC	GB:AC011088_8	124932	57		A > T	NCD			13	0	13	0	0	CAU
CTSC	GB:AC011088_8	124932	57		A > T	NCD			17	0	17	0	0	HIS
CTSC	GB:AC011088_8	124932	57		A > T	NCD			7	0	7	0	0	all

TABLE 2

Gene	Sequence	Seq Offset	Pol Position	DNA change	Peptide change	Codon	No. ind screened	Freq. of variant	No. AA	No. AB	No. BB	Ethnic group
CTSC	GB:AC011088_8	125028	153	A > G	b>T	153	47	0.9149	0	8	39	all
CTSC	GB:AC011088_8	125028	153	A > G	b>T	153	10	0.9	0	2	8	AFA
CTSC	GB:AC011088_8	125028	153	A > G	b>T	153	13	1	0	0	13	ASI
CTSC	GB:AC011088_8	125028	153	A > G	b>T	153	17	0.8235	0	6	11	CAU
CTSC	GB:AC011088_8	125028	153	A > G	b>T	153	7	1	0	0	7	HIS
CTSC	GB:AC011088_8	150261	149	G > A	L>F	9	47	0.0106	46	1	0	all
CTSC	GB:AC011088_8	150261	149	G > A	L>F	9	10	0	10	0	0	AFA
CTSC	GB:AC011088_8	150261	149	G > A	L>F	9	13	0	13	0	0	ASI
CTSC	GB:AC011088_8	150261	149	G > A	L>F	9	17	0.0294	16	1	0	CAU
CTSC	GB:AC011088_8	150261	149	G > A	L>F	9	7	0	7	0	0	HIS
CTSL	GB:AL160279_2	35919	44	C > G			47	0.0319	44	3	0	all
CTSL	GB:AL160279_2	35919	44	C > G			10	0	10	0	0	AFA
CTSL	GB:AL160279_2	35919	44	C > G			13	0	13	0	0	ASI
CTSL	GB:AL160279_2	35919	44	C > G			17	0.0294	16	1	0	CAU
CTSL	GB:AL160279_2	35919	44	C > G			7	0.1429	5	2	0	HIS
DAF	GB:AC031978_3	132041	135	C > T			47	0.0106	46	1	0	all
DAF	GB:AC031978_3	132041	135	C > T			10	0.05	9	1	0	AFA
DAF	GB:AC031978_3	132041	135	C > T			13	0	13	0	0	ASI
DAF	GB:AC031978_3	132041	135	C > T			17	0	17	0	0	CAU
DAF	GB:AC031978_3	132041	135	C > T			7	0	7	0	0	HIS
DAF	GB:AC031978_3	133893	81	A > G			47	0.0106	46	1	0	all
DAF	GB:AC031978_3	133893	81	A > G			10	0.05	9	1	0	AFA
DAF	GB:AC031978_3	133893	81	A > G			13	0	13	0	0	ASI
DAF	GB:AC031978_3	133893	81	A > G			17	0	17	0	0	CAU
DAF	GB:AC031978_3	133893	81	A > G			7	0	7	0	0	HIS
DAF	GB:AC031978_3	133974	162	C > T	C	98	47	0.0106	46	1	0	all
DAF	GB:AC031978_3	133974	162	C > T	C	98	10	0.05	9	1	0	AFA
DAF	GB:AC031978_3	133974	162	C > T	C	98	13	0	13	0	0	ASI
DAF	GB:AC031978_3	133974	162	C > T	C	98	17	0	17	0	0	CAU
DAF	GB:AC031978_3	133974	162	C > T	C	98	7	0	7	0	0	HIS
EGF	GB:AC005509	92638	222	C > T	I	88	46	0.0217	44	2	0	all
EGF	GB:AC005509	92638	222	C > T	I	88	9	0.1111	7	2	0	AFA
EGF	GB:AC005509	92638	222	C > T	I	88	13	0	13	0	0	ASI
EGF	GB:AC005509	92638	222	C > T	I	88	17	0	17	0	0	CAU
EGF	GB:AC005509	92638	222	C > T	I	88	7	0	7	0	0	HIS
EGF	GB:AC005509	92670	254	A > G	Q>R	99	46	0.0109	45	1	0	all
EGF	GB:AC005509	92670	254	A > G	Q>R	99	9	0	9	0	0	AFA
EGF	GB:AC005509	92670	254	A > G	Q>R	99	13	0	13	0	0	ASI
EGF	GB:AC005509	92670	254	A > G	Q>R	99	17	0.0294	16	1	0	CAU
EGF	GB:AC005509	92670	254	A > G	Q>R	99	7	0	7	0	0	HIS

TABLE 2

Gene	Sequence	Seq Offset	Pol	DNA change	Qualifier	Peptide change	Codon	No. Ind. screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
EGF	GB:AC005509	92670	85	A > G	MIS	Q>R	99	47	0.0106	46	1	0	all
EGF	GB:AC005509	92670	85	A > G	MIS	Q>R	99	10	0	10	0	0	AFA
EGF	GB:AC005509	92670	85	A > G	MIS	Q>R	99	13	0	13	0	0	ASI
EGF	GB:AC005509	92670	85	A > G	MIS	Q>R	99	17	0.0294	16	1	0	CAU
EGF	GB:AC005509	92670	85	A > G	MIS	Q>R	99	7	0	7	0	0	HIS
EGF	GB:AC005509	92763	178	C > T	NCD			47	0.0638	41	6	0	all
EGF	GB:AC005509	92763	178	C > T	NCD			10	0.1	8	2	0	AFA
EGF	GB:AC005509	92763	178	C > T	NCD			13	0.1538	9	4	0	ASI
EGF	GB:AC005509	92763	178	C > T	NCD			17	0	17	0	0	CAU
EGF	GB:AC005509	92763	178	C > T	NCD			7	0	7	0	0	HIS
EGF	GB:AC005509	94933	97	C > T	MIS	H>Y	151	47	0.0426	43	4	0	all
EGF	GB:AC005509	94933	97	C > T	MIS	H>Y	151	10	0.2	6	4	0	AFA
EGF	GB:AC005509	94933	97	C > T	MIS	H>Y	151	13	0	13	0	0	ASI
EGF	GB:AC005509	94933	97	C > T	MIS	H>Y	151	17	0	17	0	0	CAU
EGF	GB:AC005509	94933	97	C > T	MIS	H>Y	151	7	0	7	0	0	HIS
EGF	GB:AC005509	95444	28	G > C	MIS	D>H	186	46	0.0326	43	3	0	all
EGF	GB:AC005509	95444	28	G > C	MIS	D>H	186	9	0.1667	6	3	0	AFA
EGF	GB:AC005509	95444	28	G > C	MIS	D>H	186	13	0	13	0	0	ASI
EGF	GB:AC005509	95444	28	G > C	MIS	D>H	186	17	0	17	0	0	CAU
EGF	GB:AC005509	95444	28	G > C	MIS	D>H	186	7	0	7	0	0	HIS
EGF	GB:AC005509	96578	69	A > C	NCD			47	0.5	13	21	13	all
EGF	GB:AC005509	96578	69	A > C	NCD			10	0.65	2	3	5	AFA
EGF	GB:AC005509	96578	69	A > C	NCD			13	0.4615	4	6	3	ASI
EGF	GB:AC005509	96578	69	A > C	NCD			17	0.3529	7	8	2	CAU
EGF	GB:AC005509	96578	69	A > C	NCD			7	0.7143	0	4	3	HIS
EGF	GB:AC005509	96660	151	G > C	MIS	D>H	257	47	0.0106	46	1	0	all
EGF	GB:AC005509	96660	151	G > C	MIS	D>H	257	10	0.05	9	1	0	AFA
EGF	GB:AC005509	96660	151	G > C	MIS	D>H	257	13	0	13	0	0	ASI
EGF	GB:AC005509	96660	151	G > C	MIS	D>H	257	17	0	17	0	0	CAU
EGF	GB:AC005509	96660	151	G > C	MIS	D>H	257	7	0	7	0	0	HIS
EGF	GB:AC005509	96842	201	G > A	NCD			47	0.4894	13	22	12	all
EGF	GB:AC005509	96842	201	G > A	NCD			10	0.65	2	3	5	AFA
EGF	GB:AC005509	96842	201	G > A	NCD			13	0.3846	5	6	2	ASI
EGF	GB:AC005509	96842	201	G > A	NCD			17	0.3824	6	9	2	CAU
EGF	GB:AC005509	96842	201	G > A	NCD			7	0.7143	0	4	3	HIS
EGF	GB:AC005509	96853	212	G > A	NCD			47	0.0213	45	2	0	all
EGF	GB:AC005509	96853	212	G > A	NCD			10	0.1	8	2	0	AFA
EGF	GB:AC005509	96853	212	G > A	NCD			13	0	13	0	0	ASI
EGF	GB:AC005509	96853	212	G > A	NCD			17	0	17	0	0	CAU
EGF	GB:AC005509	96853	212	G > A	NCD			7	0	7	0	0	HIS

TABLE 2

Gene	Sequence	Seq Offset	Pol Position	DNA change	Peptide change	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
EGF	GB:AC004050	104059	127	GC > GCC		1135	46	0.0109	45	1	0	all
EGF	GB:AC004050	104059	127	GC > GCC		1135	9	0	9	0	0	AFA
EGF	GB:AC004050	104059	127	GC > GCC		1135	13	0.0385	12	1	0	ASI
EGF	GB:AC004050	104059	127	GC > GCC		1135	17	0	17	0	0	CAU
EGF	GB:AC004050	104059	127	GC > GCC		1135	7	0	7	0	0	HIS
EGF	GB:AC004050	110749	85	A > G	L	1063	47	0.0106	46	1	0	all
EGF	GB:AC004050	110749	85	A > G	L	1063	10	0.05	9	1	0	AFA
EGF	GB:AC004050	110749	85	A > G	L	1063	13	0	13	0	0	ASI
EGF	GB:AC004050	110749	85	A > G	L	1063	17	0	17	0	0	CAU
EGF	GB:AC004050	110749	85	A > G	L	1063	7	0	7	0	0	HIS
EGF	GB:AC005509	112451	117	T > C	H	365	47	0.0213	45	2	0	all
EGF	GB:AC005509	112451	117	T > C	H	365	10	0	10	0	0	AFA
EGF	GB:AC005509	112451	117	T > C	H	365	13	0	13	0	0	ASI
EGF	GB:AC005509	112451	117	T > C	H	365	17	0.0294	16	1	0	CAU
EGF	GB:AC005509	112451	117	T > C	H	365	7	0.0714	6	1	0	HIS
EGF	GB:AC005509	113396	37	T > C		365	47	0.1596	36	7	4	all
EGF	GB:AC005509	113396	37	T > C			10	0.3	5	4	1	AFA
EGF	GB:AC005509	113396	37	T > C			13	0.1538	11	0	2	ASI
EGF	GB:AC005509	113396	37	T > C			17	0	17	0	0	CAU
EGF	GB:AC005509	113396	37	T > C			7	0.3571	3	3	1	HIS
EGF	GB:AC005509	113521	88	G > A	R>K	431	47	0.0851	39	8	0	all
EGF	GB:AC005509	113521	88	G > A	R>K	431	10	0.05	9	1	0	AFA
EGF	GB:AC005509	113521	88	G > A	R>K	431	13	0.1923	8	5	0	ASI
EGF	GB:AC005509	113521	88	G > A	R>K	431	17	0.0588	15	2	0	CAU
EGF	GB:AC005509	113521	88	G > A	R>K	431	7	0	7	0	0	HIS
EGF	GB:AC005509	114696	39	C > T			47	0.0638	42	4	1	all
EGF	GB:AC005509	114696	39	C > T			10	0	10	0	0	AFA
EGF	GB:AC005509	114696	39	C > T			13	0.2308	8	4	1	ASI
EGF	GB:AC005509	114696	39	C > T			17	0	17	0	0	CAU
EGF	GB:AC005509	114696	39	C > T			7	0	7	0	0	HIS
EGF	GB:AC004050	122003	111	A > T	E>V	920	47	0.9149	0	8	39	all
EGF	GB:AC004050	122003	111	A > T	E>V	920	10	1	0	0	10	AFA
EGF	GB:AC004050	122003	111	A > T	E>V	920	13	1	0	0	13	ASI
EGF	GB:AC004050	122003	111	A > T	E>V	920	17	0.7941	0	7	10	CAU
EGF	GB:AC004050	122003	111	A > T	E>V	920	7	0.9286	0	1	6	HIS
EGF	GB:AC005509	126323	244	A > G	I>V	597	46	0.0109	45	1	0	all
EGF	GB:AC005509	126323	244	A > G	I>V	597	10	0.05	9	1	0	AFA
EGF	GB:AC005509	126323	244	A > G	I>V	597	12	0	12	0	0	ASI
EGF	GB:AC005509	126323	244	A > G	I>V	597	17	0	17	0	0	CAU
EGF	GB:AC005509	126323	244	A > G	I>V	597	7	0	7	0	0	HIS

TABLE 2

Gene	Sequence	Seq Offset	Pol	DNA change	Qualifier	Peptide change	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
EGF	GB:AC004050	126798	151	C > A	NCD			47	0.0106	46	1	0	all
EGF	GB:AC004050	126798	151	C > A	NCD			10	0.05	9	1	0	AFA
EGF	GB:AC004050	126798	151	C > A	NCD			13	0	13	0	0	ASI
EGF	GB:AC004050	126798	151	C > A	NCD			17	0	17	0	0	CAU
EGF	GB:AC004050	126798	151	C > A	NCD			7	0	7	0	0	HIS
EGF	GB:AC005509	127715	108	C > T	SIL	C	659	47	0.0426	43	4	0	all
EGF	GB:AC005509	127715	108	C > T	SIL	C	659	10	0	10	0	0	AFA
EGF	GB:AC005509	127715	108	C > T	SIL	C	659	13	0	13	0	0	ASI
EGF	GB:AC005509	127715	108	C > T	SIL	C	659	17	0.0588	15	2	0	CAU
EGF	GB:AC005509	127715	108	C > T	SIL	C	659	7	0.1429	5	2	0	HIS
EGF	GB:AC005509	131547	97	A > G	SIL	A	691	45	0.1889	28	17	0	all
EGF	GB:AC005509	131547	97	A > G	SIL	A	691	9	0.3333	3	6	0	AFA
EGF	GB:AC005509	131547	97	A > G	SIL	A	691	13	0.1923	8	5	0	ASI
EGF	GB:AC005509	131547	97	A > G	SIL	A	691	16	0.125	12	4	0	CAU
EGF	GB:AC005509	131547	97	A > G	SIL	A	691	7	0.1429	5	2	0	HIS
EGF	GB:AC005509	131598	148	G > A	MIS	M>I	708	45	0.6222	7	20	18	all
EGF	GB:AC005509	131598	148	G > A	MIS	M>I	708	9	0.8333	0	3	6	AFA
EGF	GB:AC005509	131598	148	G > A	MIS	M>I	708	13	0.6538	1	7	5	ASI
EGF	GB:AC005509	131598	148	G > A	MIS	M>I	708	16	0.4375	6	6	4	CAU
EGF	GB:AC005509	131598	148	G > A	MIS	M>I	708	7	0.7143	0	4	3	HIS
EGF	GB:AC005509	131641	191	G > C	MIS	G>R	723	45	0.0111	44	1	0	all
EGF	GB:AC005509	131641	191	G > C	MIS	G>R	723	9	0	9	0	0	AFA
EGF	GB:AC005509	131641	191	G > C	MIS	G>R	723	13	0	13	0	0	ASI
EGF	GB:AC005509	131641	191	G > C	MIS	G>R	723	16	0	16	0	0	CAU
EGF	GB:AC005509	131641	191	G > C	MIS	G>R	723	7	0.0714	6	1	0	HIS
EGF	GB:AC005509	132511	111	A > T	MIS	D>V	784	47	0.0851	39	8	0	all
EGF	GB:AC005509	132511	111	A > T	MIS	D>V	784	10	0	10	0	0	AFA
EGF	GB:AC005509	132511	111	A > T	MIS	D>V	784	13	0.2308	7	6	0	ASI
EGF	GB:AC005509	132511	111	A > T	MIS	D>V	784	17	0.0588	15	2	0	CAU
EGF	GB:AC005509	132511	111	A > T	MIS	D>V	784	7	0	7	0	0	HIS
G0S2	GB:HS28O10	52341	93	T > C	NCD			44	0.5455	13	14	17	all
G0S2	GB:HS28O10	52341	93	T > C	NCD			9	0.5556	2	4	3	AFA
G0S2	GB:HS28O10	52341	93	T > C	NCD			12	0.4167	5	4	3	ASI
G0S2	GB:HS28O10	52341	93	T > C	NCD			16	0.6875	3	4	9	CAU
G0S2	GB:HS28O10	52341	93	T > C	NCD			7	0.4286	3	2	2	HIS
GADD34	GB:AC026803_2	221941	249	G > A	SIL	R	184	47	0.0106	46	1	0	all
GADD34	GB:AC026803_2	221941	249	G > A	SIL	R	184	10	0.05	9	1	0	AFA
GADD34	GB:AC026803_2	221941	249	G > A	SIL	R	184	13	0	13	0	0	ASI
GADD34	GB:AC026803_2	221941	249	G > A	SIL	R	184	17	0	17	0	0	CAU
GADD34	GB:AC026803_2	221941	249	G > A	SIL	R	184	7	0	7	0	0	HIS

TABLE 2

Gene	Sequence	Seq Offset	Pol Position	DNA change	Qualifier	Peptide change	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
IBSP	GB:HUMBNSP03	130	107	G > A				47	0.0106	46	1	0	all
IBSP	GB:HUMBNSP03	130	107	G > A				10	0.05	9	1	0	AFA
IBSP	GB:HUMBNSP03	130	107	G > A				13	0	13	0	0	ASI
IBSP	GB:HUMBNSP03	130	107	G > A				17	0	17	0	0	CAU
IBSP	GB:HUMBNSP03	130	107	G > A				7	0	7	0	0	HIS
IHH	GB:AB018075_1	194	161	G > A	MIS	A > T	167	47	0.0106	46	1	0	all
IHH	GB:AB018075_1	194	161	G > A	MIS	A > T	167	10	0	10	0	0	AFA
IHH	GB:AB018075_1	194	161	G > A	MIS	A > T	167	13	0.0385	12	1	0	ASI
IHH	GB:AB018075_1	194	161	G > A	MIS	A > T	167	17	0	17	0	0	CAU
IHH	GB:AB018075_1	194	161	G > A	MIS	A > T	167	7	0	7	0	0	HIS
IRS1	EM:S85963	850	66	C > T	SIL	D	90	46	0.0761	40	5	1	all
IRS1	EM:S85963	850	66	C > T	SIL	D	90	10	0.2	7	2	1	AFA
IRS1	EM:S85963	850	66	C > T	SIL	D	90	13	0.0385	12	1	0	ASI
IRS1	EM:S85963	850	66	C > T	SIL	D	90	16	0.0313	15	1	0	CAU
IRS1	EM:S85963	850	66	C > T	SIL	D	90	7	0.0714	6	1	0	HIS
IRS1	EM:S85963	1285	262	G > A	SIL	G	235	45	0.1	38	5	2	all
IRS1	EM:S85963	1285	262	G > A	SIL	G	235	9	0	9	0	0	AFA
IRS1	EM:S85963	1285	262	G > A	SIL	G	235	13	0.0385	12	1	0	ASI
IRS1	EM:S85963	1285	262	G > A	SIL	G	235	17	0.1765	12	4	1	CAU
IRS1	EM:S85963	1285	262	G > A	SIL	G	235	6	0.1667	5	0	1	HIS
IRS1	EM:S85963	1285	262	G > A	SIL	G	235	47	0.0426	43	4	0	all
IRS1	EM:S85963	1285	60	G > A	SIL	G	235	10	0.05	9	1	0	AFA
IRS1	EM:S85963	1285	60	G > A	SIL	G	235	13	0.0385	12	1	0	ASI
IRS1	EM:S85963	1285	60	G > A	SIL	G	235	17	0.0294	16	1	0	CAU
IRS1	EM:S85963	1285	60	G > A	SIL	G	235	7	0.0714	6	1	0	HIS
IRS1	EM:S85963	1783	160	T > C	SIL	H	401	45	0.0111	44	1	0	all
IRS1	EM:S85963	1783	160	T > C	SIL	H	401	9	0	9	0	0	AFA
IRS1	EM:S85963	1783	160	T > C	SIL	H	401	13	0.0385	12	1	0	ASI
IRS1	EM:S85963	1783	160	T > C	SIL	H	401	16	0	16	0	0	CAU
IRS1	EM:S85963	1783	160	T > C	SIL	H	401	7	0	7	0	0	HIS
IRS1	EM:S85963	2023	234	C > T	SIL	N	481	47	0.0106	46	1	0	all
IRS1	EM:S85963	2023	234	C > T	SIL	N	481	10	0	10	0	0	AFA
IRS1	EM:S85963	2023	234	C > T	SIL	N	481	13	0	13	0	0	ASI
IRS1	EM:S85963	2023	234	C > T	SIL	N	481	17	0.0294	16	1	0	CAU
IRS1	EM:S85963	2023	234	C > T	SIL	N	481	7	0	7	0	0	HIS
IRS1	EM:S85963	2697	37	G > A	MIS	G > D	706	47	0.0106	46	1	0	all
IRS1	EM:S85963	2697	37	G > A	MIS	G > D	706	10	0	10	0	0	AFA
IRS1	EM:S85963	2697	37	G > A	MIS	G > D	706	13	0.0385	12	1	0	ASI
IRS1	EM:S85963	2697	37	G > A	MIS	G > D	706	17	0	17	0	0	CAU
IRS1	EM:S85963	2697	37	G > A	MIS	G > D	706	7	0	7	0	0	HIS

TABLE 2

Gene	Sequence	Seq Offset	Pol	DNA change	Qualifier	Peptide change	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
IRS1	EM:S85963	2995	47	A > G	SIL	A	805	47	0.3085	23	19	5	all
IRS1	EM:S85963	2995	47	A > G	SIL	A	805	10	0.45	3	5	2	AFA
IRS1	EM:S85963	2995	47	A > G	SIL	A	805	13	0.4231	4	7	2	ASI
IRS1	EM:S85963	2995	47	A > G	SIL	A	805	17	0.1471	13	3	1	CAU
IRS1	EM:S85963	2995	47	A > G	SIL	A	805	7	0.2857	3	4	0	HIS
IRS1	EM:S85963	3035	87	G > C	MIS	G>R	819	47	0.0106	46	1	0	all
IRS1	EM:S85963	3035	87	G > C	MIS	G>R	819	10	0	10	0	0	AFA
IRS1	EM:S85963	3035	87	G > C	MIS	G>R	819	13	0	13	0	0	ASI
IRS1	EM:S85963	3035	87	G > C	MIS	G>R	819	17	0.0294	16	1	0	CAU
IRS1	EM:S85963	3035	87	G > C	MIS	G>R	819	7	0	7	0	0	HIS
IRS1	EM:S85963	3262	161	G > C	SIL	P	894	47	0.0319	44	3	0	all
IRS1	EM:S85963	3262	161	G > C	SIL	P	894	10	0	10	0	0	AFA
IRS1	EM:S85963	3262	161	G > C	SIL	P	894	13	0	13	0	0	ASI
IRS1	EM:S85963	3262	161	G > C	SIL	P	894	17	0.0588	15	2	0	CAU
IRS1	EM:S85963	3262	161	G > C	SIL	P	894	7	0.0714	6	1	0	HIS
IRS1	EM:S85963	3349	248	G > A	SIL	R	923	47	0.0426	43	4	0	all
IRS1	EM:S85963	3349	248	G > A	SIL	R	923	10	0.05	9	1	0	AFA
IRS1	EM:S85963	3349	248	G > A	SIL	R	923	13	0	13	0	0	ASI
IRS1	EM:S85963	3349	248	G > A	SIL	R	923	17	0.0294	16	1	0	CAU
IRS1	EM:S85963	3349	248	G > A	SIL	R	923	7	0.1429	5	2	0	HIS
IRS1	EM:S85963	3494	155	G > A	MIS	G>R	972	47	0.0957	40	5	2	all
IRS1	EM:S85963	3494	155	G > A	MIS	G>R	972	10	0	10	0	0	AFA
IRS1	EM:S85963	3494	155	G > A	MIS	G>R	972	13	0.0385	12	1	0	ASI
IRS1	EM:S85963	3494	155	G > A	MIS	G>R	972	17	0.1765	12	4	1	CAU
IRS1	EM:S85963	3494	155	G > A	MIS	G>R	972	7	0.1429	6	0	1	HIS
IRS1	EM:S85963	3494	155	G > A	MIS	G>R	972	43	0.0116	42	1	0	all
JUN	GB:AL136985_1	8560	66	T > C	SIL	E	303	43	0.0116	9	1	0	AFA
JUN	GB:AL136985_1	8560	66	T > C	SIL	E	303	10	0.05	10	0	0	ASI
JUN	GB:AL136985_1	8560	66	T > C	SIL	E	303	10	0	10	0	0	CAU
JUN	GB:AL136985_1	8560	66	T > C	SIL	E	303	16	0	16	0	0	HIS
JUN	GB:AL136985_1	8560	66	T > C	SIL	E	303	7	0	7	0	0	all
JUN	GB:AL136985_1	8719	225	C > T	SIL	Q	250	43	0.0581	40	1	2	AFA
JUN	GB:AL136985_1	8719	225	C > T	SIL	Q	250	10	0.25	7	1	2	ASI
JUN	GB:AL136985_1	8719	225	C > T	SIL	Q	250	10	0	10	0	0	CAU
JUN	GB:AL136985_1	8719	225	C > T	SIL	Q	250	16	0	16	0	0	HIS
JUN	GB:AL136985_1	8719	225	C > T	SIL	Q	250	7	0	7	0	0	all
KJ_OA11	GB:HS425C14	42204	45	G > A	NCD			46	0.0543	42	3	1	AFA
KJ_OA11	GB:HS425C14	42204	45	G > A	NCD			9	0	9	0	0	ASI
KJ_OA11	GB:HS425C14	42204	45	G > A	NCD			13	0	13	0	0	CAU
KJ_OA11	GB:HS425C14	42204	45	G > A	NCD			17	0.0588	15	2	0	all
KJ_OA11	GB:HS425C14	42204	45	G > A	NCD			7	0.2143	5	1	1	HIS



TABLE 2

Gene	Sequence	Seq Offset	Pol	DNA change	Peptide change	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
KJ_OA11	GB:HS425C14	42294	135	G > C	L	386	46	0.0217	44	2	0	all
KJ_OA11	GB:HS425C14	42294	135	G > C	L	386	9	0.1111	7	2	0	AFA
KJ_OA11	GB:HS425C14	42294	135	G > C	L	386	13	0	13	0	0	ASI
KJ_OA11	GB:HS425C14	42294	135	G > C	L	386	17	0	17	0	0	CAU
KJ_OA11	GB:HS425C14	42294	135	G > C	L	386	7	0	7	0	0	HIS
KJ_OA11	GB:HS425C14	44297	88	T > C			47	0.1489	35	10	2	all
KJ_OA11	GB:HS425C14	44297	88	T > C			10	0.05	9	1	0	AFA
KJ_OA11	GB:HS425C14	44297	88	T > C			13	0.2692	8	3	2	ASI
KJ_OA11	GB:HS425C14	44297	88	T > C			17	0.1176	13	4	0	CAU
KJ_OA11	GB:HS425C14	44297	88	T > C			7	0.1429	5	2	0	HIS
KJ_OA11	GB:HS425C14	55697	54	C > T			45	0.0111	44	1	0	all
KJ_OA11	GB:HS425C14	55697	54	C > T			10	0.05	9	1	0	AFA
KJ_OA11	GB:HS425C14	55697	54	C > T			13	0	13	0	0	ASI
KJ_OA11	GB:HS425C14	55697	54	C > T			15	0	15	0	0	CAU
KJ_OA11	GB:HS425C14	55697	54	C > T			7	0	7	0	0	HIS
KJ_OA21	GB:HS453C12	10642	196	A > G	Y	540	47	0.8191	0	17	30	all
KJ_OA21	GB:HS453C12	10642	196	A > G	Y	540	10	0.95	0	1	9	AFA
KJ_OA21	GB:HS453C12	10642	196	A > G	Y	540	13	0.6923	0	8	5	ASI
KJ_OA21	GB:HS453C12	10642	196	A > G	Y	540	17	0.8235	0	6	11	CAU
KJ_OA21	GB:HS453C12	10642	196	A > G	Y	540	7	0.8571	0	2	5	HIS
KJ_OA21	GB:HS453C12	11999	79	C > T	T	457	47	0.2766	26	16	5	all
KJ_OA21	GB:HS453C12	11999	79	C > T	T	457	10	0.05	9	1	0	AFA
KJ_OA21	GB:HS453C12	11999	79	C > T	T	457	13	0.2692	6	7	0	ASI
KJ_OA21	GB:HS453C12	11999	79	C > T	T	457	17	0.4412	6	7	4	CAU
KJ_OA21	GB:HS453C12	11999	79	C > T	T	457	7	0.2143	5	1	1	HIS
KJ_OA21	GB:HS453C12	16925	27	CG > CCG			47	0.3085	18	29	0	all
KJ_OA21	GB:HS453C12	16925	27	CG > CCG			10	0.45	1	9	0	AFA
KJ_OA21	GB:HS453C12	16925	27	CG > CCG			13	0.1923	8	5	0	ASI
KJ_OA21	GB:HS453C12	16925	27	CG > CCG			17	0.2941	7	10	0	CAU
KJ_OA21	GB:HS453C12	16925	27	CG > CCG			7	0.3571	2	5	0	HIS
KJ_OA6	GB:AC005598	132843	24	C > T	S	48	47	0.0106	46	1	0	all
KJ_OA6	GB:AC005598	132843	24	C > T	S	48	10	0	10	0	0	AFA
KJ_OA6	GB:AC005598	132843	24	C > T	S	48	13	0.0385	12	1	0	ASI
KJ_OA6	GB:AC005598	132843	24	C > T	S	48	17	0	17	0	0	CAU
KJ_OA6	GB:AC005598	132843	24	C > T	S	48	7	0	7	0	0	HIS
KJ_OA6	GB:AC005598	132878	59	G > A	R>H	60	47	0.0106	46	1	0	all
KJ_OA6	GB:AC005598	132878	59	G > A	R>H	60	10	0	10	0	0	AFA
KJ_OA6	GB:AC005598	132878	59	G > A	R>H	60	13	0	13	0	0	ASI
KJ_OA6	GB:AC005598	132878	59	G > A	R>H	60	17	0.0294	16	1	0	CAU
KJ_OA6	GB:AC005598	132878	59	G > A	R>H	60	7	0	7	0	0	HIS

## TABLE 2

Gene	Sequence	Seg Offset	Pol Position	DNA change	Qualifier	Peptide change	Codon	No. ind. screened	Freq. of variant	No. AA	No. AB	No. BB	Ethnic group
KJ_OA6	GB:AC005598	132951	132	C > T	SIL	F	84	47	0.5426	11	21	15	all
KJ_OA6	GB:AC005598	132951	132	C > T	SIL	F	84	10	0.6	3	2	5	AFA
KJ_OA6	GB:AC005598	132951	132	C > T	SIL	F	84	13	0.3462	5	7	1	ASI
KJ_OA6	GB:AC005598	132951	132	C > T	SIL	F	84	17	0.5882	3	8	6	CAU
KJ_OA6	GB:AC005598	132951	132	C > T	SIL	F	84	7	0.7143	0	4	3	HIS
KJ_OA6	GB:AC005598	132967	148	C > T	MIS	P>S	90	47	0.4043	15	26	6	all
KJ_OA6	GB:AC005598	132967	148	C > T	MIS	P>S	90	10	0.3	5	4	1	AFA
KJ_OA6	GB:AC005598	132967	148	C > T	MIS	P>S	90	13	0.5769	1	9	3	ASI
KJ_OA6	GB:AC005598	132967	148	C > T	MIS	P>S	90	17	0.3824	6	9	2	CAU
KJ_OA6	GB:AC005598	132967	148	C > T	MIS	P>S	90	7	0.2857	3	4	0	HIS
KJ_OA6	GB:AC005598	133103	147	G > T	MIS	G>V	135	47	0.0426	45	0	2	all
KJ_OA6	GB:AC005598	133103	147	G > T	MIS	G>V	135	10	0.1	9	0	1	AFA
KJ_OA6	GB:AC005598	133103	147	G > T	MIS	G>V	135	13	0	13	0	0	ASI
KJ_OA6	GB:AC005598	133103	147	G > T	MIS	G>V	135	17	0.0588	16	0	1	CAU
KJ_OA6	GB:AC005598	133103	147	G > T	MIS	G>V	135	7	0	7	0	0	HIS
KJ_OA6	GB:AC005598	133481	188	C > T	NCD			46	0.0109	45	1	0	all
KJ_OA6	GB:AC005598	133481	188	C > T	NCD			9	0	9	0	0	AFA
KJ_OA6	GB:AC005598	133481	188	C > T	NCD			13	0	13	0	0	ASI
KJ_OA6	GB:AC005598	133481	188	C > T	NCD			17	0	17	0	0	CAU
KJ_OA6	GB:AC005598	133481	188	C > T	NCD			7	0.0714	6	1	0	HIS
MMP1	GB:HSU78045	4517	43	A > G	SIL	D	433	47	0.0213	45	2	0	all
MMP1	GB:HSU78045	4517	43	A > G	SIL	D	433	10	0	10	0	0	AFA
MMP1	GB:HSU78045	4517	43	A > G	SIL	D	433	13	0.0769	11	2	0	ASI
MMP1	GB:HSU78045	4517	43	A > G	SIL	D	433	17	0	17	0	0	CAU
MMP1	GB:HSU78045	4517	43	A > G	SIL	D	433	7	0	7	0	0	HIS
MMP1	GB:HSU78045	4661	187	CATG > CG	NCD			47	0.0106	46	1	0	all
MMP1	GB:HSU78045	4661	187	CATG > CG	NCD			10	0.05	9	1	0	AFA
MMP1	GB:HSU78045	4661	187	CATG > CG	NCD			13	0	13	0	0	ASI
MMP1	GB:HSU78045	4661	187	CATG > CG	NCD			17	0	17	0	0	CAU
MMP1	GB:HSU78045	4661	187	CATG > CG	NCD			7	0	7	0	0	HIS
MMP1	GB:HSU78045	4677	203	G > A	NCD			47	0.0638	44	0	3	all
MMP1	GB:HSU78045	4677	203	G > A	NCD			10	0.1	9	0	1	AFA
MMP1	GB:HSU78045	4677	203	G > A	NCD			13	0.1538	11	0	2	ASI
MMP1	GB:HSU78045	4677	203	G > A	NCD			17	0	17	0	0	CAU
MMP1	GB:HSU78045	4677	203	G > A	NCD			7	0	7	0	0	HIS
MMP1	GB:HSU78045	5198	169	A > G	MIS	S>P	382	46	0.0109	45	1	0	all
MMP1	GB:HSU78045	5198	169	A > G	MIS	S>P	382	10	0.05	9	1	0	AFA
MMP1	GB:HSU78045	5198	169	A > G	MIS	S>P	382	13	0	13	0	0	ASI
MMP1	GB:HSU78045	5198	169	A > G	MIS	S>P	382	16	0	16	0	0	CAU
MMP1	GB:HSU78045	5198	169	A > G	MIS	S>P	382	7	0	7	0	0	HIS

TABLE 2

Gene	Sequence	Seq Offset	Pol Position	DNA change	Peptide change	Codon	No ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
MMP1	GB:HSU78045	6586	237	T > C		NCD	46	0.413	18	18	10	all
MMP1	GB:HSU78045	6586	237	T > C		NCD	10	0.25	6	3	1	AFA
MMP1	GB:HSU78045	6586	237	T > C		NCD	13	0.7308	0	7	6	ASI
MMP1	GB:HSU78045	6586	237	T > C		NCD	16	0.3125	9	4	3	CAU
MMP1	GB:HSU78045	6586	237	T > C		NCD	7	0.2857	3	4	0	HIS
MMP1	GB:HSU78045	9056	54	C > T	A	277	46	0.0652	40	6	0	all
MMP1	GB:HSU78045	9056	54	C > T	A	277	9	0.1111	7	2	0	AFA
MMP1	GB:HSU78045	9056	54	C > T	A	277	13	0.0385	12	1	0	ASI
MMP1	GB:HSU78045	9056	54	C > T	A	277	17	0.0882	14	3	0	CAU
MMP1	GB:HSU78045	9056	54	C > T	A	277	7	0	7	0	0	HIS
MMP1	GB:HSU78045	9120	118	A > G		NCD	46	0.7826	10	0	36	all
MMP1	GB:HSU78045	9120	118	A > G		NCD	9	0.7778	2	0	7	AFA
MMP1	GB:HSU78045	9120	118	A > G		NCD	13	0.8462	2	0	11	ASI
MMP1	GB:HSU78045	9120	118	A > G		NCD	17	0.7647	4	0	13	CAU
MMP1	GB:HSU78045	9120	118	A > G		NCD	7	0.7143	2	0	5	HIS
MMP1	GB:HSU78045	9120	26	A > G		NCD	46	0.0326	43	3	0	all
MMP1	GB:HSU78045	9120	26	A > G		NCD	10	0.1	8	2	0	AFA
MMP1	GB:HSU78045	9120	26	A > G		NCD	12	0	12	0	0	ASI
MMP1	GB:HSU78045	9120	26	A > G		NCD	17	0.0294	16	1	0	CAU
MMP1	GB:HSU78045	9120	26	A > G		NCD	7	0	7	0	0	HIS
MMP1	GB:HSU78045	9126	124	G > A		NCD	46	0.0435	42	4	0	all
MMP1	GB:HSU78045	9126	124	G > A		NCD	9	0	9	0	0	AFA
MMP1	GB:HSU78045	9126	124	G > A		NCD	13	0.0385	12	1	0	ASI
MMP1	GB:HSU78045	9126	124	G > A		NCD	17	0.0294	16	1	0	CAU
MMP1	GB:HSU78045	9126	124	G > A		NCD	7	0.1429	5	2	0	HIS
MMP1	GB:HSU78045	9126	32	G > A		NCD	46	0.0109	45	1	0	all
MMP1	GB:HSU78045	9126	32	G > A		NCD	10	0	10	0	0	AFA
MMP1	GB:HSU78045	9126	32	G > A		NCD	12	0	12	0	0	ASI
MMP1	GB:HSU78045	9126	32	G > A		NCD	17	0	17	0	0	CAU
MMP1	GB:HSU78045	9126	32	G > A		NCD	7	0.0714	6	1	0	HIS
MMP1	GB:HSU78045	9205	111	T > C		NCD	46	0.0109	45	1	0	all
MMP1	GB:HSU78045	9205	111	T > C		NCD	10	0.05	9	1	0	AFA
MMP1	GB:HSU78045	9205	111	T > C		NCD	12	0	12	0	0	ASI
MMP1	GB:HSU78045	9205	111	T > C		NCD	17	0	17	0	0	CAU
MMP1	GB:HSU78045	9205	111	T > C		NCD	7	0	7	0	0	HIS
MMP1	GB:HSU78045	9247	153	T > C		NCD	46	0.0543	42	3	1	all
MMP1	GB:HSU78045	9247	153	T > C		NCD	10	0.15	8	1	1	AFA
MMP1	GB:HSU78045	9247	153	T > C		NCD	12	0.0417	11	1	0	ASI
MMP1	GB:HSU78045	9247	153	T > C		NCD	17	0.0294	16	1	0	CAU
MMP1	GB:HSU78045	9247	153	T > C		NCD	7	0	7	0	0	HIS

TABLE 2

Gene	Sequence	Seq Offset	Pol Position	DNA change	Qualifier	Peptide change	Codon	No. ind. screened	Freq. of variant	No. AA	No. AB	No. BB	Ethnic group
MMP1	GB:HSU78045	9365	271	G > T	MIS	H>N	228	46	0.0109	45	1	0	all
MMP1	GB:HSU78045	9365	271	G > T	MIS	H>N	228	10	0	10	0	0	AFA
MMP1	GB:HSU78045	9365	271	G > T	MIS	H>N	228	12	0	12	0	0	ASI
MMP1	GB:HSU78045	9365	271	G > T	MIS	H>N	228	17	0.0294	16	1	0	CAU
MMP1	GB:HSU78045	9365	271	G > T	MIS	H>N	228	7	0	7	0	0	HIS
MMP1	GB:HSU78045	11105	177	C > T	SIL	G	105	47	0.0532	43	3	1	all
MMP1	GB:HSU78045	11105	177	C > T	SIL	G	105	10	0.15	8	1	1	AFA
MMP1	GB:HSU78045	11105	177	C > T	SIL	G	105	13	0.0385	12	1	0	ASI
MMP1	GB:HSU78045	11105	177	C > T	SIL	G	105	17	0.0294	16	1	0	CAU
MMP1	GB:HSU78045	11105	177	C > T	SIL	G	105	7	0	7	0	0	HIS
MMP13	GB:AP000789_1	141614	30	C > G	NCD			46	0.0109	45	1	0	all
MMP13	GB:AP000789_1	141614	30	C > G	NCD			9	0.0556	8	1	0	AFA
MMP13	GB:AP000789_1	141614	30	C > G	NCD			13	0	13	0	0	ASI
MMP13	GB:AP000789_1	141614	30	C > G	NCD			17	0	17	0	0	CAU
MMP13	GB:AP000789_1	141614	30	C > G	NCD			7	0	7	0	0	HIS
MMP13	GB:AP000789_1	147095	141	A > G	MIS	H>R	192	43	0.0116	42	1	0	all
MMP13	GB:AP000789_1	147095	141	A > G	MIS	H>R	192	9	0	9	0	0	AFA
MMP13	GB:AP000789_1	147095	141	A > G	MIS	H>R	192	12	0	12	0	0	ASI
MMP13	GB:AP000789_1	147095	141	A > G	MIS	H>R	192	15	0.0333	14	1	0	CAU
MMP13	GB:AP000789_1	147095	141	A > G	MIS	H>R	192	7	0	7	0	0	HIS
MMP13	GB:AP000789_1	157231	114	G > C	MIS	G>R	239	45	0.0111	44	1	0	all
MMP13	GB:AP000789_1	157231	114	G > C	MIS	G>R	239	10	0	10	0	0	AFA
MMP13	GB:AP000789_1	157231	114	G > C	MIS	G>R	239	12	0	12	0	0	ASI
MMP13	GB:AP000789_1	157231	114	G > C	MIS	G>R	239	16	0.0313	15	1	0	CAU
MMP13	GB:AP000789_1	157231	114	G > C	MIS	G>R	239	7	0	7	0	0	HIS
MMP13	GB:AP000789_1	157325	208	A > G	MIS	D>G	270	45	0.0222	44	0	1	all
MMP13	GB:AP000789_1	157325	208	A > G	MIS	D>G	270	10	0.1	9	0	1	AFA
MMP13	GB:AP000789_1	157325	208	A > G	MIS	D>G	270	12	0	12	0	0	ASI
MMP13	GB:AP000789_1	157325	208	A > G	MIS	D>G	270	16	0	16	0	0	CAU
MMP13	GB:AP000789_1	157325	208	A > G	MIS	D>G	270	7	0	7	0	0	HIS
MMP14	GB:AL133448_3	137049	28	TTA > TA	NCD			46	0.9022	0	9	37	all
MMP14	GB:AL133448_3	137049	28	TTA > TA	NCD			10	0.9	0	2	8	AFA
MMP14	GB:AL133448_3	137049	28	TTA > TA	NCD			13	1	0	0	13	ASI
MMP14	GB:AL133448_3	137049	28	TTA > TA	NCD			16	0.8438	0	5	11	CAU
MMP14	GB:AL133448_3	137049	28	TTA > TA	NCD			7	0.8571	0	2	5	HIS
MMP14	GB:AL133448_3	137049	28	TTA > TA	NCD			47	0.0426	43	4	0	all
MMP14	GB:AL133448_3	138406	47	G > A	NCD			10	0.2	6	4	0	AFA
MMP14	GB:AL133448_3	138406	47	G > A	NCD			13	0	13	0	0	ASI
MMP14	GB:AL133448_3	138406	47	G > A	NCD			17	0	17	0	0	CAU
MMP14	GB:AL133448_3	138406	47	G > A	NCD			7	0	7	0	0	HIS

TABLE 2

Gene	Sequence	Seq Offset	Pol Position	DNA change	Qualifier	Peptide change	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
MMP14	GB:AL133448_3	138560	201	C > G	SIL	P	259	47	0.0745	40	7	0	all
MMP14	GB:AL133448_3	138560	201	C > G	SIL	P	259	10	0.05	9	1	0	AFA
MMP14	GB:AL133448_3	138560	201	C > G	SIL	P	259	13	0.0769	11	2	0	ASI
MMP14	GB:AL133448_3	138560	201	C > G	SIL	P	259	17	0.0588	15	2	0	CAU
MMP14	GB:AL133448_3	138560	201	C > G	SIL	P	259	7	0.1429	5	2	0	HIS
MMP14	GB:AL133448_3	138653	104	G > A	NCD			47	0.0106	46	1	0	all
MMP14	GB:AL133448_3	138653	104	G > A	NCD			10	0	10	0	0	AFA
MMP14	GB:AL133448_3	138653	104	G > A	NCD			13	0	13	0	0	ASI
MMP14	GB:AL133448_3	138653	104	G > A	NCD			17	0	17	0	0	CAU
MMP14	GB:AL133448_3	138653	104	G > A	NCD			7	0.0714	6	1	0	HIS
MMP14	GB:AL133448_3	138639	101	G > A	MIS	M>I	355	46	0.0217	44	2	0	all
MMP14	GB:AL133448_3	138639	101	G > A	MIS	M>I	355	9	0	9	0	0	AFA
MMP14	GB:AL133448_3	138639	101	G > A	MIS	M>I	355	13	0	13	0	0	ASI
MMP14	GB:AL133448_3	138639	101	G > A	MIS	M>I	355	17	0.0294	16	1	0	CAU
MMP14	GB:AL133448_3	138639	101	G > A	MIS	M>I	355	7	0.0714	6	1	0	HIS
MMP14	GB:AL133448_3	139981	54	C > T	SIL	F	429	47	0.0319	44	3	0	all
MMP14	GB:AL133448_3	139981	54	C > T	SIL	F	429	10	0.1	8	2	0	AFA
MMP14	GB:AL133448_3	139981	54	C > T	SIL	F	429	13	0	13	0	0	ASI
MMP14	GB:AL133448_3	139981	54	C > T	SIL	F	429	17	0	17	0	0	CAU
MMP14	GB:AL133448_3	139981	54	C > T	SIL	F	429	7	0.0714	6	1	0	HIS
MMP14	GB:AL133448_3	139986	59	G > A	MIS	R>H	431	47	0.0106	46	1	0	all
MMP14	GB:AL133448_3	139986	59	G > A	MIS	R>H	431	10	0	10	0	0	AFA
MMP14	GB:AL133448_3	139986	59	G > A	MIS	R>H	431	13	0.0385	12	1	0	ASI
MMP14	GB:AL133448_3	139986	59	G > A	MIS	R>H	431	17	0	17	0	0	CAU
MMP14	GB:AL133448_3	139986	59	G > A	MIS	R>H	431	7	0	7	0	0	HIS
MMP2	GB:AC012182_3	158292	70	G > A	SIL	S	520	46	0.087	40	4	2	all
MMP2	GB:AC012182_3	158292	70	G > A	SIL	S	520	9	0.3889	4	3	2	AFA
MMP2	GB:AC012182_3	158292	70	G > A	SIL	S	520	13	0	13	0	0	ASI
MMP2	GB:AC012182_3	158292	70	G > A	SIL	S	520	17	0	17	0	0	CAU
MMP2	GB:AC012182_3	158292	70	G > A	SIL	S	520	7	0.0714	6	1	0	HIS
MMP2	GB:AC012182_3	158292	70	G > A	SIL	S	520	47	0.1702	39	0	8	all
MMP2	GB:AC012182_3	158292	70	G > A	SIL	S	520	10	0	10	0	0	AFA
MMP2	GB:AC012182_3	158292	70	G > A	SIL	S	520	13	0.3846	8	0	5	ASI
MMP2	GB:AC012182_3	158292	70	G > A	SIL	S	520	17	0.0588	16	0	1	CAU
MMP2	GB:AC012182_3	158292	70	G > A	SIL	S	520	7	0.2857	5	0	2	HIS
MMP2	GB:AC012182_3	158292	70	G > A	SIL	S	520	46	0.0109	45	1	0	all
MMP3	EM:HSU78045	52375	108	T > C	SIL	T	400	10	0.05	9	1	0	AFA
MMP3	EM:HSU78045	52375	108	T > C	SIL	T	400	12	0	12	0	0	ASI
MMP3	EM:HSU78045	52375	108	T > C	SIL	T	400	17	0	17	0	0	CAU
MMP3	EM:HSU78045	52375	108	T > C	SIL	T	400	7	0	7	0	0	HIS

TABLE 2

Gene	Sequence	Seq Offset	Pol Position	DNA change	Qualifier	Peptide change	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
MMP3	EM:HSU78045	52411	144	G>A	SIL	I	388	46	0.0109	45	1	0	all
MMP3	EM:HSU78045	52411	144	G>A	SIL	I	388	10	0.05	9	1	0	AFA
MMP3	EM:HSU78045	52411	144	G>A	SIL	I	388	12	0	12	0	0	ASI
MMP3	EM:HSU78045	52411	144	G>A	SIL	I	388	17	0	17	0	0	CAU
MMP3	EM:HSU78045	52411	144	G>A	SIL	I	388	7	0	7	0	0	HIS
MMP3	EM:HSU78045	53771	27	T>A	NCD			47	0.0638	42	4	1	all
MMP3	EM:HSU78045	53771	27	T>A	NCD			10	0	10	0	0	AFA
MMP3	EM:HSU78045	53771	27	T>A	NCD			13	0.0385	12	1	0	ASI
MMP3	EM:HSU78045	53771	27	T>A	NCD			17	0.1471	13	3	1	CAU
MMP3	EM:HSU78045	53771	27	T>A	NCD			7	0	7	0	0	HIS
MMP3	EM:HSU78045	54077	133	C>G	NCD			45	0.0111	44	1	0	all
MMP3	EM:HSU78045	54077	133	C>G	NCD			9	0	9	0	0	AFA
MMP3	EM:HSU78045	54077	133	C>G	NCD			13	0	13	0	0	ASI
MMP3	EM:HSU78045	54077	133	C>G	NCD			16	0.0313	15	1	0	CAU
MMP3	EM:HSU78045	54077	133	C>G	NCD			7	0	7	0	0	HIS
MMP3	EM:HSU78045	54187	81	C>T	NCD			47	0.0106	46	1	0	all
MMP3	EM:HSU78045	54187	81	C>T	NCD			10	0.05	9	1	0	AFA
MMP3	EM:HSU78045	54187	81	C>T	NCD			13	0	13	0	0	ASI
MMP3	EM:HSU78045	54187	81	C>T	NCD			17	0	17	0	0	CAU
MMP3	EM:HSU78045	54187	81	C>T	NCD			7	0	7	0	0	HIS
MMP3	EM:HSU78045	56119	272	C>T	NCD			46	0.1739	37	2	7	all
MMP3	EM:HSU78045	56119	272	C>T	NCD			10	0.2	8	0	2	AFA
MMP3	EM:HSU78045	56119	272	C>T	NCD			13	0.0385	12	1	0	ASI
MMP3	EM:HSU78045	56119	272	C>T	NCD			16	0.2188	12	1	3	CAU
MMP3	EM:HSU78045	56119	272	C>T	NCD			7	0.2857	5	0	2	HIS
MMP3	EM:HSU78045	56507	173	G>C	SIL	T	102	46	0.0217	45	0	1	all
MMP3	EM:HSU78045	56507	173	G>C	SIL	T	102	10	0	10	0	0	AFA
MMP3	EM:HSU78045	56507	173	G>C	SIL	T	102	13	0	13	0	0	ASI
MMP3	EM:HSU78045	56507	173	G>C	SIL	T	102	17	0	17	0	0	CAU
MMP3	EM:HSU78045	56507	173	G>C	SIL	T	102	6	0.1667	5	0	1	HIS
MMP3	EM:HSU78045	56525	191	G>A	SIL	D	96	46	0.5435	1	40	5	all
MMP3	EM:HSU78045	56525	191	G>A	SIL	D	96	10	0.65	0	7	3	AFA
MMP3	EM:HSU78045	56525	191	G>A	SIL	D	96	13	0.5	0	13	0	ASI
MMP3	EM:HSU78045	56525	191	G>A	SIL	D	96	17	0.5588	0	15	2	CAU
MMP3	EM:HSU78045	56525	191	G>A	SIL	D	96	6	0.4167	1	5	0	HIS
MMP3	EM:HSU78045	56680	122	C>T	MIS	E>K	45	47	0.2021	37	1	9	all
MMP3	EM:HSU78045	56680	122	C>T	MIS	E>K	45	10	0.35	6	1	3	AFA
MMP3	EM:HSU78045	56680	122	C>T	MIS	E>K	45	13	0.1538	11	0	2	ASI
MMP3	EM:HSU78045	56680	122	C>T	MIS	E>K	45	17	0.2353	13	0	4	CAU
MMP3	EM:HSU78045	56680	122	C>T	MIS	E>K	45	7	0	7	0	0	HIS

TABLE 2

Gene	Sequence	Seq Offset	Pol Position	DNA change	Qualifier	Peptide change	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
MMP9	GB:HUMIVCOL13	87	73	G>A	SIL	V	694	47	0.1915	29	18	0	all
MMP9	GB:HUMIVCOL13	87	73	G>A	SIL	V	694	10	0.15	7	3	0	AFA
MMP9	GB:HUMIVCOL13	87	73	G>A	SIL	V	694	13	0.1923	8	5	0	ASI
MMP9	GB:HUMIVCOL13	87	73	G>A	SIL	V	694	17	0.1765	11	6	0	CAU
MMP9	GB:HUMIVCOL13	87	73	G>A	SIL	V	694	7	0.2857	3	4	0	HIS
MMP9	GB:HUMIVCOL13	132	118	C>T	NCD			47	0.7766	3	15	29	all
MMP9	GB:HUMIVCOL13	132	118	C>T	NCD			10	0.85	0	3	7	AFA
MMP9	GB:HUMIVCOL13	132	118	C>T	NCD			13	0.8077	0	5	8	ASI
MMP9	GB:HUMIVCOL13	132	118	C>T	NCD			17	0.7941	1	5	11	CAU
MMP9	GB:HUMIVCOL13	132	118	C>T	NCD			7	0.5714	2	2	3	HIS
MMP9	GB:HUMIVCOL01	677	111	C>T	MIS	A>V	20	47	0.0106	46	1	0	all
MMP9	GB:HUMIVCOL01	677	111	C>T	MIS	A>V	20	10	0	10	0	0	AFA
MMP9	GB:HUMIVCOL01	677	111	C>T	MIS	A>V	20	13	0.0385	12	1	0	ASI
MMP9	GB:HUMIVCOL01	677	111	C>T	MIS	A>V	20	17	0	17	0	0	CAU
MMP9	GB:HUMIVCOL01	677	111	C>T	MIS	A>V	20	7	0	7	0	0	HIS
MMP9	GB:HUMIVCOL01	677	92	C>T	MIS	A>V	20	47	0.0106	46	1	0	all
MMP9	GB:HUMIVCOL01	677	92	C>T	MIS	A>V	20	10	0	10	0	0	AFA
MMP9	GB:HUMIVCOL01	677	92	C>T	MIS	A>V	20	13	0.0385	12	1	0	ASI
MMP9	GB:HUMIVCOL01	677	92	C>T	MIS	A>V	20	17	0	17	0	0	CAU
MMP9	GB:HUMIVCOL01	677	92	C>T	MIS	A>V	20	7	0	7	0	0	HIS
MSF	GB:AL133553_7	190505	30	A>C	MIS	D>A	220	47	0.0213	45	2	0	all
MSF	GB:AL133553_7	190505	30	A>C	MIS	D>A	220	10	0.05	9	1	0	AFA
MSF	GB:AL133553_7	190505	30	A>C	MIS	D>A	220	13	0	13	0	0	ASI
MSF	GB:AL133553_7	190505	30	A>C	MIS	D>A	220	17	0	17	0	0	CAU
MSF	GB:AL133553_7	190505	30	A>C	MIS	D>A	220	7	0.0714	6	1	0	HIS
MSF	GB:AL133553_7	190559	84	C>T	MIS	T>M	238	47	0.0213	45	2	0	all
MSF	GB:AL133553_7	190559	84	C>T	MIS	T>M	238	10	0	10	0	0	AFA
MSF	GB:AL133553_7	190559	84	C>T	MIS	T>M	238	13	0	13	0	0	ASI
MSF	GB:AL133553_7	190559	84	C>T	MIS	T>M	238	17	0.0588	15	2	0	CAU
MSF	GB:AL133553_7	190559	84	C>T	MIS	T>M	238	7	0	7	0	0	HIS
MSF	GB:AL133553_7	190755	162	G>A	SIL	K	303	43	0.0581	38	5	0	all
MSF	GB:AL133553_7	190755	162	G>A	SIL	K	303	7	0.0714	6	1	0	AFA
MSF	GB:AL133553_7	190755	162	G>A	SIL	K	303	13	0.1154	10	3	0	ASI
MSF	GB:AL133553_7	190755	162	G>A	SIL	K	303	17	0	17	0	0	CAU
MSF	GB:AL133553_7	190755	162	G>A	SIL	K	303	6	0.0833	5	1	0	HIS
MSF	GB:AL133553_7	193235	32	A>G	MIS	N>S	1130	47	0.0106	46	1	0	all
MSF	GB:AL133553_7	193235	32	A>G	MIS	N>S	1130	10	0.05	9	1	0	AFA
MSF	GB:AL133553_7	193235	32	A>G	MIS	N>S	1130	13	0	13	0	0	ASI
MSF	GB:AL133553_7	193235	32	A>G	MIS	N>S	1130	17	0	17	0	0	CAU
MSF	GB:AL133553_7	193235	32	A>G	MIS	N>S	1130	7	0	7	0	0	HIS

TABLE 2

Gene	Sequence	Seq. Offset	Pos.	DNA change	Qualifier	Peptide change	Codon	No. ind. screened	Freq. of variant	No. AA	No. AB	No. BB	Ethnic group
MSF	GB:AL133553_7	193258	55	A > G	MIS	M>V	1138	47	0.0106	46	1	0	all
MSF	GB:AL133553_7	193258	55	A > G	MIS	M>V	1138	10	0	10	0	0	AFA
MSF	GB:AL133553_7	193258	55	A > G	MIS	M>V	1138	13	0.0385	12	1	0	ASI
MSF	GB:AL133553_7	193258	55	A > G	MIS	M>V	1138	17	0	17	0	0	CAU
MSF	GB:AL133553_7	193258	55	A > G	MIS	M>V	1138	7	0	7	0	0	HIS
NOG	GB:AC005553	145585	63	G > A	SIL	R	206	47	0.0106	46	1	0	all
NOG	GB:AC005553	145585	63	G > A	SIL	R	206	10	0	10	0	0	AFA
NOG	GB:AC005553	145585	63	G > A	SIL	R	206	13	0	13	0	0	ASI
NOG	GB:AC005553	145585	63	G > A	SIL	R	206	17	0.0294	16	1	0	CAU
NOG	GB:AC005553	145585	63	G > A	SIL	R	206	7	0	7	0	0	HIS
NOTCH3	GB:AC004663_1	5567	126	T > C	NCD			47	0.9468	0	5	42	all
NOTCH3	GB:AC004663_1	5567	126	T > C	NCD			10	0.8	0	4	6	AFA
NOTCH3	GB:AC004663_1	5567	126	T > C	NCD			13	1	0	0	13	ASI
NOTCH3	GB:AC004663_1	5567	126	T > C	NCD			17	1	0	0	17	CAU
NOTCH3	GB:AC004663_1	5567	126	T > C	NCD			7	0.9286	0	1	6	HIS
NOTCH3	GB:AC004663_1	21959	43	A > T	NCD			49	0.5204	0	47	2	all
NOTCH3	GB:AC004663_1	21959	43	A > T	NCD			10	0.5	0	10	0	AFA
NOTCH3	GB:AC004663_1	21959	43	A > T	NCD			13	0.5	0	13	0	ASI
NOTCH3	GB:AC004663_1	21959	43	A > T	NCD			17	0.5	0	17	0	CAU
NOTCH3	GB:AC004663_1	21959	43	A > T	NCD			7	0.5	0	7	0	HIS
NOTCH3	GB:AC004663_1	21959	43	A > T	NCD			2				0	UNK
NOTCH3	GB:AC004663_1	22353	245	C > T	MIS	V>M	1143	47	0.0319	44	3	0	all
NOTCH3	GB:AC004663_1	22353	245	C > T	MIS	V>M	1143	10	0.05	9	1	0	AFA
NOTCH3	GB:AC004663_1	22353	245	C > T	MIS	V>M	1143	13	0	13	0	0	ASI
NOTCH3	GB:AC004663_1	22353	245	C > T	MIS	V>M	1143	17	0	17	0	0	CAU
NOTCH3	GB:AC004663_1	22353	245	C > T	MIS	V>M	1143	7	0.1429	5	2	0	HIS
NOTCH3	GB:AC004663_1	23922	149	C > G	MIS	A>P	980	47	0.0426	43	4	0	all
NOTCH3	GB:AC004663_1	23922	149	C > G	MIS	A>P	980	10	0.1	8	2	0	AFA
NOTCH3	GB:AC004663_1	23922	149	C > G	MIS	A>P	980	13	0	13	0	0	ASI
NOTCH3	GB:AC004663_1	23922	149	C > G	MIS	A>P	980	17	0	17	0	0	CAU
NOTCH3	GB:AC004663_1	23922	149	C > G	MIS	A>P	980	7	0.1429	5	2	0	HIS
NOTCH3	GB:AC004663_1	23922	149	C > G	MIS	A>P	980	47	0.0213	45	2	0	all
NOTCH3	GB:AC004663_1	24045	272	T > C	NCD			10	0.05	9	1	0	AFA
NOTCH3	GB:AC004663_1	24045	272	T > C	NCD			13	0	13	0	0	ASI
NOTCH3	GB:AC004663_1	24045	272	T > C	NCD			17	0	17	0	0	CAU
NOTCH3	GB:AC004663_1	24045	272	T > C	NCD			7	0.1429	5	2	0	HIS
NOTCH3	GB:AC004663_1	24045	272	T > C	NCD			47	0.0213	45	2	0	all
NOTCH3	GB:AC004663_1	24045	272	T > C	NCD			10	0.05	9	1	0	AFA
NOTCH3	GB:AC004663_1	24045	272	T > C	NCD			13	0	13	0	0	ASI
NOTCH3	GB:AC004663_1	24045	272	T > C	NCD			17	0	17	0	0	CAU
NOTCH3	GB:AC004663_1	24045	272	T > C	NCD			7	0.0714	6	1	0	HIS
NOTCH3	GB:AC004663_1	28173	175	C > T	MIS	R>H	727	47	0.0106	46	1	0	all
NOTCH3	GB:AC004663_1	28173	175	C > T	MIS	R>H	727	10	0.05	9	1	0	AFA
NOTCH3	GB:AC004663_1	28173	175	C > T	MIS	R>H	727	13	0	13	0	0	ASI
NOTCH3	GB:AC004663_1	28173	175	C > T	MIS	R>H	727	17	0	17	0	0	CAU



TABLE 2

Gene	Sequence	Seq Offset	Pol Position	DNA change	Qualifier	Peptide change	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
NOTCH3	GB:AC004663_1	28173	175	C > T	MIS	R>H	727	7	0	7	0	0	HIS
NOTCH3	GB:AC004663_1	28749	174	C > T	MIS	R>H	640	47	0.0426	43	4	0	all
NOTCH3	GB:AC004663_1	28749	174	C > T	MIS	R>H	640	10	0.2	6	4	0	AFA
NOTCH3	GB:AC004663_1	28749	174	C > T	MIS	R>H	640	13	0	13	0	0	ASI
NOTCH3	GB:AC004663_1	28749	174	C > T	MIS	R>H	640	17	0	17	0	0	CAU
NOTCH3	GB:AC004663_1	28749	174	C > T	MIS	R>H	640	7	0	7	0	0	HIS
NOTCH3	GB:AC004663_1	29997	50	G > C	NCD			46	0.022	43	2	0	all
NOTCH3	GB:AC004663_1	29997	50	G > C	NCD			10	0.1	8	2	0	AFA
NOTCH3	GB:AC004663_1	29997	50	G > C	NCD			13	0	13	0	0	ASI
NOTCH3	GB:AC004663_1	29997	50	G > C	NCD			16	0	16	0	0	CAU
NOTCH3	GB:AC004663_1	29997	50	G > C	NCD			7	0	6	0	0	HIS
PDNP1	98092911024828	201	22	A > G				47	0.0106	46	1	0	all
PDNP1	98092911024828	201	22	A > G				10	0	10	0	0	AFA
PDNP1	98092911024828	201	22	A > G				13	0.0385	12	1	0	ASI
PDNP1	98092911024828	201	22	A > G				17	0	17	0	0	CAU
PDNP1	98092911024828	201	22	A > G				7	0	7	0	0	HIS
PDNP1	98092910591328	232	78	C > T				47	0.0213	45	2	0	all
PDNP1	98092910591328	232	78	C > T				10	0	10	0	0	AFA
PDNP1	98092910591328	232	78	C > T				13	0	13	0	0	ASI
PDNP1	98092910591328	232	78	C > T				17	0.0588	15	2	0	CAU
PDNP1	98092910591328	232	78	C > T				7	0	7	0	0	HIS
PDNP1	98092911013628	364	82	C > T				47	0.0106	46	1	0	all
PDNP1	98092911013628	364	82	C > T				10	0.05	9	1	0	AFA
PDNP1	98092911013628	364	82	C > T				13	0	13	0	0	ASI
PDNP1	98092911013628	364	82	C > T				17	0	17	0	0	CAU
PDNP1	98092911013628	364	82	C > T				7	0	7	0	0	HIS
PLA2G2A	GB:AL358253_1	51295	29	G > A	NCD			47	0.1277	35	12	0	all
PLA2G2A	GB:AL358253_1	51295	29	G > A	NCD			10	0.15	7	3	0	AFA
PLA2G2A	GB:AL358253_1	51295	29	G > A	NCD			13	0.0385	12	1	0	ASI
PLA2G2A	GB:AL358253_1	51295	29	G > A	NCD			17	0.2059	10	7	0	CAU
PLA2G2A	GB:AL358253_1	51295	29	G > A	NCD			7	0.0714	6	1	0	HIS
PLA2G2A	GB:AL358253_1	51364	98	G > A	SIL	Y	44	47	0.1277	35	12	0	all
PLA2G2A	GB:AL358253_1	51364	98	G > A	SIL	Y	44	10	0.15	7	3	0	AFA
PLA2G2A	GB:AL358253_1	51364	98	G > A	SIL	Y	44	13	0.0385	12	1	0	ASI
PLA2G2A	GB:AL358253_1	51364	98	G > A	SIL	Y	44	17	0.2059	10	7	0	CAU
PLA2G2A	GB:AL358253_1	51364	98	G > A	SIL	Y	44	7	0.0714	6	1	0	HIS
PLA2G2A	GB:AL358253_1	51400	134	C > G	SIL	T	32	47	0.0213	45	2	0	all
PLA2G2A	GB:AL358253_1	51400	134	C > G	SIL	T	32	10	0	10	0	0	AFA
PLA2G2A	GB:AL358253_1	51400	134	C > G	SIL	T	32	13	0.0385	12	1	0	ASI
PLA2G2A	GB:AL358253_1	51400	134	C > G	SIL	T	32	17	0.0294	16	1	0	CAU

TABLE 2

Gene	Sequence	Seq Offset	Pol Position	DNA change	Peptide change	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
PLA2G2A	GB:AL358253_1	51400	134	C > G	T	32	7	0	7	0	0	HIS
PLA2G2A	GB:AL358253_1	51454	188	G > A	G	14	47	0.0106	46	1	0	all
PLA2G2A	GB:AL358253_1	51454	188	G > A	G	14	10	0	10	0	0	AFA
PLA2G2A	GB:AL358253_1	51454	188	G > A	G	14	13	0.0385	12	1	0	ASI
PLA2G2A	GB:AL358253_1	51454	188	G > A	G	14	17	0	17	0	0	CAU
PLA2G2A	GB:AL358253_1	51454	188	G > A	G	14	7	0	7	0	0	HIS
PLA2G2A	GB:AL358253_1	51503	237	C > T	NCD		47	0.5	1	45	1	all
PLA2G2A	GB:AL358253_1	51503	237	C > T	NCD		10	0.5	0	10	0	AFA
PLA2G2A	GB:AL358253_1	51503	237	C > T	NCD		13	0.5385	0	12	1	ASI
PLA2G2A	GB:AL358253_1	51503	237	C > T	NCD		17	0.4706	1	16	0	CAU
PLA2G2A	GB:AL358253_1	51503	237	C > T	NCD		7	0.5	0	7	0	HIS
PPP1R5	GB:AC020691_2	106523	122	T > G	D>E	110	47	0.0319	44	3	0	all
PPP1R5	GB:AC020691_2	106523	122	T > G	D>E	110	10	0.1	8	2	0	AFA
PPP1R5	GB:AC020691_2	106523	122	T > G	D>E	110	13	0	13	0	0	ASI
PPP1R5	GB:AC020691_2	106523	122	T > G	D>E	110	17	0	17	0	0	CAU
PPP1R5	GB:AC020691_2	106523	122	T > G	D>E	110	7	0.0714	6	1	0	HIS
PRSS11	GB:AF157623_1	44762	42	G > T	NCD		47	0.0106	46	1	0	all
PRSS11	GB:AF157623_1	44762	42	G > T	NCD		10	0.05	9	1	0	AFA
PRSS11	GB:AF157623_1	44762	42	G > T	NCD		13	0	13	0	0	ASI
PRSS11	GB:AF157623_1	44762	42	G > T	NCD		17	0	17	0	0	CAU
PRSS11	GB:AF157623_1	44762	42	G > T	NCD		7	0	7	0	0	HIS
PRSS11	GB:AF157623_1	45470	66	C > T	I	251	47	0.0426	43	4	0	all
PRSS11	GB:AF157623_1	45470	66	C > T	I	251	10	0.05	9	1	0	AFA
PRSS11	GB:AF157623_1	45470	66	C > T	I	251	13	0	13	0	0	ASI
PRSS11	GB:AF157623_1	45470	66	C > T	I	251	17	0.0882	14	3	0	CAU
PRSS11	GB:AF157623_1	45470	66	C > T	I	251	7	0	7	0	0	HIS
PRSS11	GB:AF157623_1	45587	183	C > T	NCD		47	0.2447	27	17	3	all
PRSS11	GB:AF157623_1	45587	183	C > T	NCD		10	0.15	7	3	0	AFA
PRSS11	GB:AF157623_1	45587	183	C > T	NCD		13	0.3846	5	6	2	ASI
PRSS11	GB:AF157623_1	45587	183	C > T	NCD		17	0.1765	11	6	0	CAU
PRSS11	GB:AF157623_1	45587	183	C > T	NCD		7	0.2857	4	2	1	HIS
PRSS11	GB:AF157623_1	62541	83	G > A	NCD		47	0.0319	44	3	0	all
PRSS11	GB:AF157623_1	62541	83	G > A	NCD		10	0.1	8	2	0	AFA
PRSS11	GB:AF157623_1	62541	83	G > A	NCD		13	0	13	0	0	ASI
PRSS11	GB:AF157623_1	62541	83	G > A	NCD		17	0	17	0	0	CAU
PRSS11	GB:AF157623_1	62541	83	G > A	NCD		7	0.0714	6	1	0	HIS
PRSS11	GB:AF157623_1	62545	87	G > A	NCD		47	0.0106	46	1	0	all
PRSS11	GB:AF157623_1	62545	87	G > A	NCD		10	0	10	0	0	AFA
PRSS11	GB:AF157623_1	62545	87	G > A	NCD		13	0	13	0	0	ASI
PRSS11	GB:AF157623_1	62545	87	G > A	NCD		17	0.0294	16	1	0	CAU

TABLE 2

Gene	Sequence	Seq Offset	Pol Position	DNA change	Qualifier	Peptide change	Codon	No. ind. screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
PRSS11	GB:AF157623_1	62545	87	G > A	NCD			7	0	7	0	0	HIS
PRSS11	GB:AF157623_1	63355	186	GTTT > T	NCD			47	0.3085	18	29	0	all
PRSS11	GB:AF157623_1	63355	186	GTTT > T	NCD			10	0.35	3	7	0	AFA
PRSS11	GB:AF157623_1	63355	186	GTTT > T	NCD			13	0.2308	7	6	0	ASI
PRSS11	GB:AF157623_1	63355	186	GTTT > T	NCD			17	0.3235	6	11	0	CAU
PRSS11	GB:AF157623_1	63355	186	GTTT > T	NCD			7	0.3571	2	5	0	HIS
PTGS2	GB:HUMPTGS2	3050	141	G > C	SIL	V	102	46	0.0543	41	5	0	all
PTGS2	GB:HUMPTGS2	3050	141	G > C	SIL	V	102	10	0	10	0	0	AFA
PTGS2	GB:HUMPTGS2	3050	141	G > C	SIL	V	102	13	0	13	0	0	ASI
PTGS2	GB:HUMPTGS2	3050	141	G > C	SIL	V	102	16	0.1563	11	5	0	CAU
PTGS2	GB:HUMPTGS2	3050	141	G > C	SIL	V	102	7	0	7	0	0	HIS
PTGS2	GB:HUMPTGS2	3090	181	C > T	NCD			46	0.0109	45	1	0	all
PTGS2	GB:HUMPTGS2	3090	181	C > T	NCD			10	0	10	0	0	AFA
PTGS2	GB:HUMPTGS2	3090	181	C > T	NCD			13	0.0385	12	1	0	ASI
PTGS2	GB:HUMPTGS2	3090	181	C > T	NCD			16	0	16	0	0	CAU
PTGS2	GB:HUMPTGS2	3090	181	C > T	NCD			7	0	7	0	0	HIS
PTGS2	GB:HUMPTGS2	3174	265	G > C	NCD			46	0.0217	44	2	0	all
PTGS2	GB:HUMPTGS2	3174	265	G > C	NCD			10	0.05	9	1	0	AFA
PTGS2	GB:HUMPTGS2	3174	265	G > C	NCD			13	0.0385	12	1	0	ASI
PTGS2	GB:HUMPTGS2	3174	265	G > C	NCD			16	0	16	0	0	CAU
PTGS2	GB:HUMPTGS2	3174	265	G > C	NCD			7	0	7	0	0	HIS
PTGS2	GB:HUMPTGS2	3793	177	C > T	SIL	S	132	47	0.0106	46	1	0	all
PTGS2	GB:HUMPTGS2	3793	177	C > T	SIL	S	132	10	0	10	0	0	AFA
PTGS2	GB:HUMPTGS2	3793	177	C > T	SIL	S	132	13	0.0385	12	1	0	ASI
PTGS2	GB:HUMPTGS2	3793	177	C > T	SIL	S	132	17	0	17	0	0	CAU
PTGS2	GB:HUMPTGS2	3793	177	C > T	SIL	S	132	7	0	7	0	0	HIS
PTGS2	GB:HUMPTGS2	3829	213	T > C	SIL	D	144	47	0.0106	46	1	0	all
PTGS2	GB:HUMPTGS2	3829	213	T > C	SIL	D	144	10	0	10	0	0	AFA
PTGS2	GB:HUMPTGS2	3829	213	T > C	SIL	D	144	13	0.0385	12	1	0	ASI
PTGS2	GB:HUMPTGS2	3829	213	T > C	SIL	D	144	17	0	17	0	0	CAU
PTGS2	GB:HUMPTGS2	3829	213	T > C	SIL	D	144	7	0	7	0	0	HIS
PTGS2	GB:HUMPTGS2	5605	110	G > A	NCD			45	0.0111	44	1	0	all
PTGS2	GB:HUMPTGS2	5605	110	G > A	NCD			10	0	10	0	0	AFA
PTGS2	GB:HUMPTGS2	5605	110	G > A	NCD			13	0	13	0	0	ASI
PTGS2	GB:HUMPTGS2	5605	110	G > A	NCD			15	0.0333	14	1	0	CAU
PTGS2	GB:HUMPTGS2	5605	110	G > A	NCD			7	0	7	0	0	HIS
PTGS2	GB:HUMPTGS2	5676	181	ATTTT > T	NCD			45	0.0111	44	1	0	all
PTGS2	GB:HUMPTGS2	5676	181	ATTTT > T	NCD			10	0	10	0	0	AFA
PTGS2	GB:HUMPTGS2	5676	181	ATTTT > T	NCD			13	0	13	0	0	ASI
PTGS2	GB:HUMPTGS2	5676	181	ATTTT > T	NCD			15	0.0333	14	1	0	CAU

TABLE 2

Gene	Sequence	Seq Offset	Pol Position	DNA change	Peptide change	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
PTGS2	GB:HUMPTGS2	5676	181	TATTT > T			7	0	7	0	0	HIS
PTGS2	GB:HUMPTGS2	6249	106	G > A	SIL	335	47	0.0106	46	1	0	all
PTGS2	GB:HUMPTGS2	6249	106	G > A	SIL	335	10	0	10	0	0	AFA
PTGS2	GB:HUMPTGS2	6249	106	G > A	SIL	335	13	0.0385	12	1	0	ASI
PTGS2	GB:HUMPTGS2	6249	106	G > A	SIL	335	17	0	17	0	0	CAU
PTGS2	GB:HUMPTGS2	6249	106	G > A	SIL	335	7	0	7	0	0	HIS
PTGS2	GB:HUMPTGS2	6444	94	G > A	SIL	400	47	0.0106	46	1	0	all
PTGS2	GB:HUMPTGS2	6444	94	G > A	SIL	400	10	0	10	0	0	AFA
PTGS2	GB:HUMPTGS2	6444	94	G > A	SIL	400	13	0.0385	12	1	0	ASI
PTGS2	GB:HUMPTGS2	6444	94	G > A	SIL	400	17	0	17	0	0	CAU
PTGS2	GB:HUMPTGS2	6444	94	G > A	SIL	400	7	0	7	0	0	HIS
PTGS2	GB:HUMPTGS2	6453	103	T > C	SIL	403	47	0.0106	46	1	0	all
PTGS2	GB:HUMPTGS2	6453	103	T > C	SIL	403	10	0.05	9	1	0	AFA
PTGS2	GB:HUMPTGS2	6453	103	T > C	SIL	403	13	0	13	0	0	ASI
PTGS2	GB:HUMPTGS2	6453	103	T > C	SIL	403	17	0	17	0	0	CAU
PTGS2	GB:HUMPTGS2	6453	103	T > C	SIL	403	7	0	7	0	0	HIS
PTGS2	GB:HUMPTGS2	7581	32	T > C	NCD		47	0.0106	46	1	0	all
PTGS2	GB:HUMPTGS2	7581	32	T > C	NCD		10	0.05	9	1	0	AFA
PTGS2	GB:HUMPTGS2	7581	32	T > C	NCD		13	0	13	0	0	ASI
PTGS2	GB:HUMPTGS2	7581	32	T > C	NCD		17	0	17	0	0	CAU
PTGS2	GB:HUMPTGS2	7581	32	T > C	NCD		7	0	7	0	0	HIS
PTGS2	GB:HUMPTGS2	7763	214	T > C	MIS	511	47	0.0106	46	1	0	all
PTGS2	GB:HUMPTGS2	7763	214	T > C	MIS	511	10	0.05	9	1	0	AFA
PTGS2	GB:HUMPTGS2	7763	214	T > C	MIS	511	13	0	13	0	0	ASI
PTGS2	GB:HUMPTGS2	7763	214	T > C	MIS	511	17	0	17	0	0	CAU
PTGS2	GB:HUMPTGS2	7763	214	T > C	MIS	511	7	0	7	0	0	HIS
PTGS2	GB:HUMPTGS2	7986	89	C > A	SIL	585	47	0.0106	46	1	0	all
PTGS2	GB:HUMPTGS2	7986	89	C > A	SIL	585	10	0.05	9	1	0	AFA
PTGS2	GB:HUMPTGS2	7986	89	C > A	SIL	585	13	0	13	0	0	ASI
PTGS2	GB:HUMPTGS2	7986	89	C > A	SIL	585	17	0	17	0	0	CAU
PTGS2	GB:HUMPTGS2	7986	89	C > A	SIL	585	7	0	7	0	0	HIS
PTHR1	GB:HSPHPRH3	104	29	G > A	SIL	72	47	0.0106	46	1	0	all
PTHR1	GB:HSPHPRH3	104	29	G > A	SIL	72	10	0	10	0	0	AFA
PTHR1	GB:HSPHPRH3	104	29	G > A	SIL	72	13	0	13	0	0	ASI
PTHR1	GB:HSPHPRH3	104	29	G > A	SIL	72	17	0.0294	16	1	0	CAU
PTHR1	GB:HSPHPRH3	104	29	G > A	SIL	72	7	0	7	0	0	HIS
PTHR1	GB:HSPHPRH8	311	207	T > C	SIL	463	46	0.6413	6	21	19	all
PTHR1	GB:HSPHPRH8	311	207	T > C	SIL	463	9	0.8333	0	3	6	AFA
PTHR1	GB:HSPHPRH8	311	207	T > C	SIL	463	13	0.5769	3	5	5	ASI
PTHR1	GB:HSPHPRH8	311	207	T > C	SIL	463	17	0.6471	1	10	6	CAU

TABLE 2

Gene	Sequence	Seq Offset	Position	Pol	DNA change	Qualifier	Peptide change	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
PTHR1	GB:HSPTPHRH8	311	207		T > C	SIL	N	463	7	0.5	2	3	2	HIS
SOD2	EM:S77127	1183	47		C > T	MIS	A>V	16	45	0.6111	7	21	17	all
SOD2	EM:S77127	1183	47		C > T	MIS	A>V	16	10	0.55	2	5	3	AFA
SOD2	EM:S77127	1183	47		C > T	MIS	A>V	16	12	0.9167	0	2	10	ASI
SOD2	EM:S77127	1183	47		C > T	MIS	A>V	16	16	0.5	4	8	4	CAU
SOD2	EM:S77127	1183	47		C > T	MIS	A>V	16	7	0.4286	1	6	0	HIS
SOD2	EM:S77127	1456	209		A > C	NCD			45	0.0111	44	1	0	all
SOD2	EM:S77127	1456	209		A > C	NCD			10	0.05	9	1	0	AFA
SOD2	EM:S77127	1456	209		A > C	NCD			12	0	12	0	0	ASI
SOD2	EM:S77127	1456	209		A > C	NCD			17	0	17	0	0	CAU
SOD2	EM:S77127	1456	209		A > C	NCD			6	0	6	0	0	HIS
SOX9	GB:AC007461_8	62482	48		G > A	SIL	H	169	45	0.0667	39	6	0	all
SOX9	GB:AC007461_8	62482	48		G > A	SIL	H	169	10	0	10	0	0	AFA
SOX9	GB:AC007461_8	62482	48		G > A	SIL	H	169	13	0	13	0	0	ASI
SOX9	GB:AC007461_8	62482	48		G > A	SIL	H	169	16	0.125	12	4	0	CAU
SOX9	GB:AC007461_8	62482	48		G > A	SIL	H	169	6	0.1667	4	2	0	HIS
TIMP1	GB:HS230G1	17434	221		G > A	SIL	I	158	47	0.0532	42	5	0	all
TIMP1	GB:HS230G1	17434	221		G > A	SIL	I	158	10	0	10	0	0	AFA
TIMP1	GB:HS230G1	17434	221		G > A	SIL	I	158	13	0.0769	11	2	0	ASI
TIMP1	GB:HS230G1	17434	221		G > A	SIL	I	158	17	0.0294	16	1	0	CAU
TIMP1	GB:HS230G1	17434	221		G > A	SIL	I	158	7	0.1429	5	2	0	HIS
TIMP1	GB:HS230G1	18389	148		A > G	SIL	F	124	47	0.5106	20	6	21	all
TIMP1	GB:HS230G1	18389	148		A > G	SIL	F	124	10	0.75	2	1	7	AFA
TIMP1	GB:HS230G1	18389	148		A > G	SIL	F	124	13	0.5385	5	2	6	ASI
TIMP1	GB:HS230G1	18389	148		A > G	SIL	F	124	17	0.3235	11	1	5	CAU
TIMP1	GB:HS230G1	18389	148		A > G	SIL	F	124	7	0.5714	2	2	3	HIS
TIMP1	GB:HS230G1	18495	254		C > G	NCD			47	0.5106	20	6	21	all
TIMP1	GB:HS230G1	18495	254		C > G	NCD			10	0.75	2	1	7	AFA
TIMP1	GB:HS230G1	18495	254		C > G	NCD			13	0.5385	5	2	6	ASI
TIMP1	GB:HS230G1	18495	254		C > G	NCD			17	0.3235	11	1	5	CAU
TIMP1	GB:HS230G1	18495	254		C > G	NCD			7	0.5714	2	2	3	HIS
TIMP1	GB:HS230G1	18711	173		G > A	SIL	P	87	47	0.0106	46	1	0	all
TIMP1	GB:HS230G1	18711	173		G > A	SIL	P	87	10	0.05	9	1	0	AFA
TIMP1	GB:HS230G1	18711	173		G > A	SIL	P	87	13	0	13	0	0	ASI
TIMP1	GB:HS230G1	18711	173		G > A	SIL	P	87	17	0	17	0	0	CAU
TIMP1	GB:HS230G1	18711	173		G > A	SIL	P	87	7	0	7	0	0	HIS
TIMP2	GB:U44383_1	155	146		G > A	SIL	S	101	47	0.1064	37	10	0	all
TIMP2	GB:U44383_1	155	146		G > A	SIL	S	101	10	0	10	0	0	AFA
TIMP2	GB:U44383_1	155	146		G > A	SIL	S	101	13	0.0769	11	2	0	ASI
TIMP2	GB:U44383_1	155	146		G > A	SIL	S	101	17	0.1471	12	5	0	CAU

TABLE 2

Gene	Sequence	Seq Offset	Pol	DNA change	Qualifier	Peptide change	Codon	No. Ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
TIMP1	GB:HS230G1	18495	254	C > G	NCD			17	0.3235	11	1	5	CAU
TIMP1	GB:HS230G1	18495	254	C > G	NCD			7	0.5714	2	2	3	HIS
TIMP1	GB:HS230G1	18711	173	G > A	SIL	P	87	47	0.0106	46	1	0	all
TIMP1	GB:HS230G1	18711	173	G > A	SIL	P	87	17	0.05	9	1	0	AFA
TIMP1	GB:HS230G1	18711	173	G > A	SIL	P	87	13	0	13	0	0	ASI
TIMP1	GB:HS230G1	18711	173	G > A	SIL	P	87	17	0	17	0	0	CAU
TIMP1	GB:HS230G1	18711	173	G > A	SIL	P	87	7	0	7	0	0	HIS
TIMP2	GB:U44383_1	155	146	G > A	SIL	S	101	47	0.1064	37	10	0	all
TIMP2	GB:U44383_1	155	146	G > A	SIL	S	101	10	0	10	0	0	AFA
TIMP2	GB:U44383_1	155	146	G > A	SIL	S	101	13	0.0769	11	2	0	ASI
TIMP2	GB:U44383_1	155	146	G > A	SIL	S	101	17	0.1471	12	5	0	CAU
TIMP2	GB:U44383_1	155	146	G > A	SIL	S	101	7	0.2143	4	3	0	HIS
TNFAIP6	GB:AC009311_1	140934	117	G > A	SPL			47	0.0213	45	2	0	all
TNFAIP6	GB:AC009311_1	140934	117	G > A	SPL			10	0.1	8	2	0	AFA
TNFAIP6	GB:AC009311_1	140934	117	G > A	SPL			13	0	13	0	0	ASI
TNFAIP6	GB:AC009311_1	140934	117	G > A	SPL			17	0	17	0	0	CAU
TNFAIP6	GB:AC009311_1	140934	117	G > A	SPL			7	0	7	0	0	HIS
TNFAIP6	GB:AC009311_1	140942	125	A > T	NCD			47	0.0213	45	2	0	all
TNFAIP6	GB:AC009311_1	140942	125	A > T	NCD			10	0.1	8	2	0	AFA
TNFAIP6	GB:AC009311_1	140942	125	A > T	NCD			13	0	13	0	0	ASI
TNFAIP6	GB:AC009311_1	140942	125	A > T	NCD			17	0	17	0	0	CAU
TNFAIP6	GB:AC009311_1	140942	125	A > T	NCD			7	0	7	0	0	HIS
TNFAIP6	GB:AC009311_1	144773	83	A > G	MIS	Q>R	144	47	0.1702	33	12	2	all
TNFAIP6	GB:AC009311_1	144773	83	A > G	MIS	Q>R	144	10	0.25	6	3	1	AFA
TNFAIP6	GB:AC009311_1	144773	83	A > G	MIS	Q>R	144	13	0.1538	9	4	0	ASI
TNFAIP6	GB:AC009311_1	144773	83	A > G	MIS	Q>R	144	17	0.1471	13	3	1	CAU
TNFAIP6	GB:AC009311_1	144773	83	A > G	MIS	Q>R	144	7	0.1429	5	2	0	HIS
TNFAIP6	GB:AC009311_1	148229	47	T > C	NCD			45	0.0778	38	7	0	all
TNFAIP6	GB:AC009311_1	148229	47	T > C	NCD			10	0.1	8	2	0	AFA
TNFAIP6	GB:AC009311_1	148229	47	T > C	NCD			12	0.1667	8	4	0	ASI
TNFAIP6	GB:AC009311_1	148229	47	T > C	NCD			16	0	16	0	0	CAU
TNFAIP6	GB:AC009311_1	148229	47	T > C	NCD			7	0.0714	6	1	0	HIS
TNFAIP6	GB:AC009311_1	148245	63	T > C	NCD			45	0.0778	38	7	0	all
TNFAIP6	GB:AC009311_1	148245	63	T > C	NCD			10	0.1	8	2	0	AFA
TNFAIP6	GB:AC009311_1	148245	63	T > C	NCD			12	0.1667	8	4	0	ASI
TNFAIP6	GB:AC009311_1	148245	63	T > C	NCD			16	0	16	0	0	CAU
TNFAIP6	GB:AC009311_1	148245	63	T > C	NCD			7	0.0714	6	1	0	HIS
TNFRSF1B	GB:E15270_1	503	80	C > T	NCD			47	0.0851	39	8	0	all
TNFRSF1B	GB:E15270_1	503	80	C > T	NCD			10	0.1	8	2	0	AFA
TNFRSF1B	GB:E15270_1	503	80	C > T	NCD			13	0	13	0	0	ASI
TNFRSF1B	GB:E15270_1	503	80	C > T	NCD			17	0.1471	12	5	0	CAU
TNFRSF1B	GB:E15270_1	503	80	C > T	NCD			7	0.0714	6	1	0	HIS
TNFRSF1B	GB:E15270_1	4499	46	C > T	NCD			47	0.0745	40	7	0	all

TABLE 2

Gene	Sequence	Seq Offset	Pol Position	DNA change	Qualifier	Peptide change	Codon	No. ind screened	Freq of variant	No. AA	No. AB	No. BB	Ethnic group
TNFRSF11B	GB:E15270_1	4499	46	C > T	NCD			10	0.05	9	1	0	AFA
TNFRSF11B	GB:E15270_1	4499	46	C > T	NCD			13	0.0769	11	2	0	ASI
TNFRSF11B	GB:E15270_1	4499	46	C > T	NCD			17	0.0882	14	3	0	CAU
TNFRSF11B	GB:E15270_1	4499	46	C > T	NCD			7	0.0714	6	1	0	HIS
TNFRSF11B	GB:E15270_1	4661	159	C > T	SIL	S	176	47	0.0106	46	1	0	all
TNFRSF11B	GB:E15270_1	4661	159	C > T	SIL	S	176	10	0.05	9	1	0	AFA
TNFRSF11B	GB:E15270_1	4661	159	C > T	SIL	S	176	13	0	13	0	0	ASI
TNFRSF11B	GB:E15270_1	4661	159	C > T	SIL	S	176	17	0	17	0	0	CAU
TNFRSF11B	GB:E15270_1	4661	159	C > T	SIL	S	176	7	0	7	0	0	HIS
TNFRSF11B	GB:E15270_1	4749	247	TCTG > TG	NCD			47	0.1702	33	12	2	all
TNFRSF11B	GB:E15270_1	4749	247	TCTG > TG	NCD			10	0.5	2	6	2	AFA
TNFRSF11B	GB:E15270_1	4749	247	TCTG > TG	NCD			13	0	13	0	0	ASI
TNFRSF11B	GB:E15270_1	4749	247	TCTG > TG	NCD			17	0.1471	12	5	0	CAU
TNFRSF11B	GB:E15270_1	4749	247	TCTG > TG	NCD			7	0.0714	6	1	0	HIS
TNFRSF11B	GB:E15270_1	6599	44	G > A	NCD			47	0.0106	46	1	0	all
TNFRSF11B	GB:E15270_1	6599	44	G > A	NCD			10	0.05	9	1	0	AFA
TNFRSF11B	GB:E15270_1	6599	44	G > A	NCD			13	0	13	0	0	ASI
TNFRSF11B	GB:E15270_1	6599	44	G > A	NCD			17	0	17	0	0	CAU
TNFRSF11B	GB:E15270_1	6599	44	G > A	NCD			7	0	7	0	0	HIS
TNFRSF11B	GB:E15270_1	6837	103	G > A	SIL	E	228	43	0.0349	40	3	0	all
TNFRSF11B	GB:E15270_1	6837	103	G > A	SIL	E	228	8	0.125	6	2	0	AFA
TNFRSF11B	GB:E15270_1	6837	103	G > A	SIL	E	228	13	0	13	0	0	ASI
TNFRSF11B	GB:E15270_1	6837	103	G > A	SIL	E	228	15	0.0333	14	1	0	CAU
TNFRSF11B	GB:E15270_1	6837	103	G > A	SIL	E	228	7	0	7	0	0	HIS

Column 1: Gene Name

Column 2: Sequence

Column 3: Position offset in link object

Column 4: Position of the polymorphism

Column 5: DNA change

Column 6: Change type: NCD (non-coding), SIL (silent), STP (stop), FSH (frame shift), MIS (missense).

Column 7: The peptide change as a result of the polymorphism. One letter amino acid abbreviations are used. A \* indicates a stop codon.

Column 8: This is the codon number which contains the DNA change.

Column 9: The number is the total number of individuals that have been assigned a genotype for the DNA change.

Column 10: Frequency of variant. This figure is calculated as follows: B alleles/(A+B alleles). The figure is specific to those samples tested.

Column 11: Total number of individuals with homozygote wild type genotype.

Column 12: Number AB, Total number of individuals with heterozygote genotype.

Column 13: Number BB, Total number of individuals with homozygote mutant genotype.

Column 14: Ethnic group. The different ethnic groups used. CAU= Caucasian, AFC= African, ASI= Asian, HIS= Hispanic, UNK= unknown.

What is claimed is:

1. A method of determining susceptibility of an individual to joint space narrowing and/or osteophyte development and/or joint pain comprising identifying whether the individual has at least one polymorphism in a polynucleotide encoding at least one of the proteins listed in Table 1.

2. The method of claim 1, wherein said proteins listed in Table 1 are selected from the group consisting of bone morphogenic protein 2 (BMP2), cartilage intermediate layer protein (CILP), cartilage oligomeric matrix protein (COMP), tissue inhibitor of metalloproteinase 1 (TIMP1), tetranectin (TNA), matrix metalloproteinase 3 (MMP3), and prostaglandin-endoperoxide synthase 2 (PTGS2).

3. The method of claim 1, wherein the joint space narrowing and/or osteophyte development and/or joint pain is associated with a disease.

4. The method of claim 3 wherein the disease is osteoarthritis.

5. The method of claim 1 where at least one of the polymorphisms is selected from the polymorphisms listed in Table 1.

6. The method of claim 1 comprising contacting a sample from the individual with a specific binding agent for the polymorphism and determining whether the agent binds to the polymorphism.

7. The method of claim 1 where the polymorphism in the polynucleotide is determined for more than one allele of the individual.

8. A method for modulating the susceptibility of an individual to joint space narrowing and/or osteophyte development and/or joint pain, comprising identifying the individual by the method of claim 1 and administering to the individual a composition comprising an effective amount of an agent which modulates said susceptibility.

9. The method of claim 8, wherein the joint space narrowing and/or osteophyte development and/or joint pain is associated with a disease.



10. The method of claim 9 wherein the disease is osteoarthritis.

11. A polynucleotide encoding a protein listed in Table 1 having at least one polymorphism in the polynucleotide selected from the group of polymorphisms listed in Table 1 for the polynucleotide.

5

12. A fragment of a polynucleotide encoding a protein selected from Table 1 having at least one polymorphism in the fragment selected from the group of polymorphisms listed in Table 1.

13. A fragment of claim 12 having a length of 8 to 100 nucleotides.

10

14. A fragment of claim 12 having a length of 8 to 30 nucleotides.

15. A fragment of claim 12 having a length of 9 to 15 nucleotides.

15

16. A method of identifying an agent for modulating susceptibility of an individual to joint space narrowing and/or osteophyte development and/or joint pain comprising:

a) contacting a test agent with a polypeptide or a polynucleotide encoding the polypeptide selected from the list of Table 1 having at least one of the polymorphisms selected from the list of Table 1,

20

b) determining whether the agent is capable of binding to the polypeptide or polynucleotide encoding the polypeptide, and

c) determining whether the activity or expression of the polypeptide or polynucleotide encoding the polypeptide is modulated.

25

17. A method of formulating a composition comprising

a) identifying an agent for modulating the susceptibility of an individual to joint space narrowing and/or osteophyte development and/or joint pain by the method of claim 16, and

b) formulating the agent with a carrier or diluent.

30

18. An agent identified by the method of claim 16.

19. A composition for modulating the susceptibility of an individual to joint space narrowing and/or osteophyte development and/or joint pain comprising an agent according to claim 18 and a carrier.

5 20. A method comprising using an agent of claim 18 in the manufacture of a medicament for modulating susceptibility to joint space narrowing and/or osteophyte development and/or joint pain.

21. A probe, primer or antibody which is capable of selectively detecting a polymorphism listed in Table 1 which is associated with susceptibility to joint space narrowing and/or osteophyte  
10 development and/or joint pain.

22. A vector comprising the polynucleotide of claim 11.

23. A host cell line comprising the vector of claim 22.

15 24. A nonhuman animal which is transgenic for the polynucleotide of claim 11.

25. A cell line comprising the polynucleotide of claim 11.

20 26. A method of using a cell line of claim 25 to screen for an agent for diagnosis of an individual having susceptibility to joint space narrowing and/or osteophyte development and/or joint pain.

27. A method of using a nonhuman animal of claim 24 to screen for an agent for diagnosis of  
25 an individual having susceptibility to joint space narrowing and/or osteophyte development and/or joint pain.

28. A kit for diagnosis of an individual having susceptibility to joint space narrowing and/or osteophyte development and/or joint pain comprising an agent for detection of the polynucleotide of  
30 claim 11.

29. The kit of claim 28 further comprising instruction for use of said agent for detection of said polynucleotide.

30. A kit for diagnosis of an individual having susceptibility to joint space narrowing and/or osteophyte development and/or joint pain comprising an agent for detection of the fragment of a polynucleotide of claim 12.

5           31. The kit of claim 30 further comprising instructions for use of said agent for detection of said fragment.

32. A kit for diagnosis of an individual having susceptibility to joint space narrowing and/or osteophyte development and/or joint pain comprising the probe, primer or antibody of claim 21.

10

33. The kit of claim 32 further comprising instructions for use of said probe, primer or antibody.

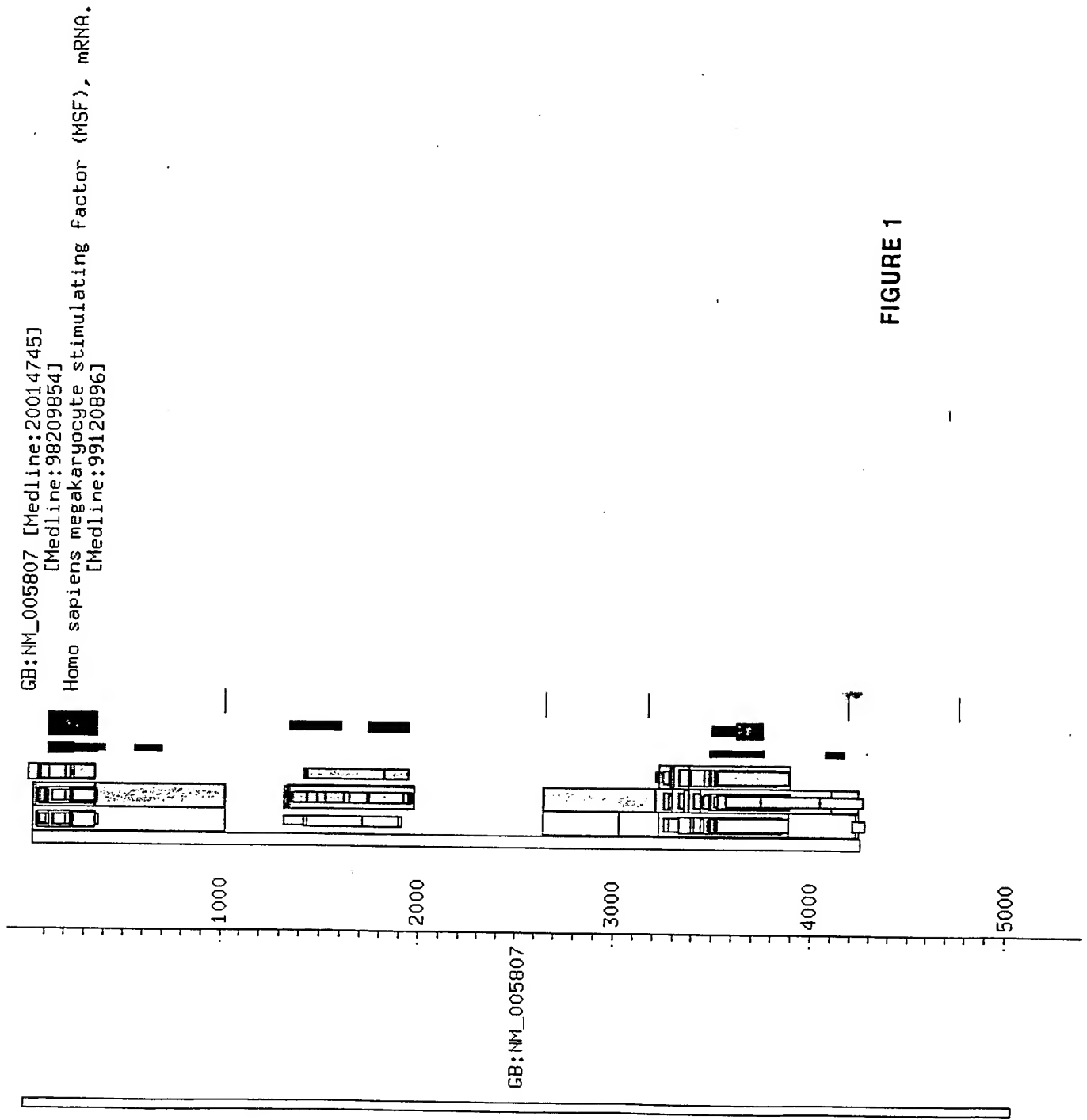


FIGURE 1

GB:AL133553\_7  
Homo sapiens chromosome 1 clone GS1-174L6

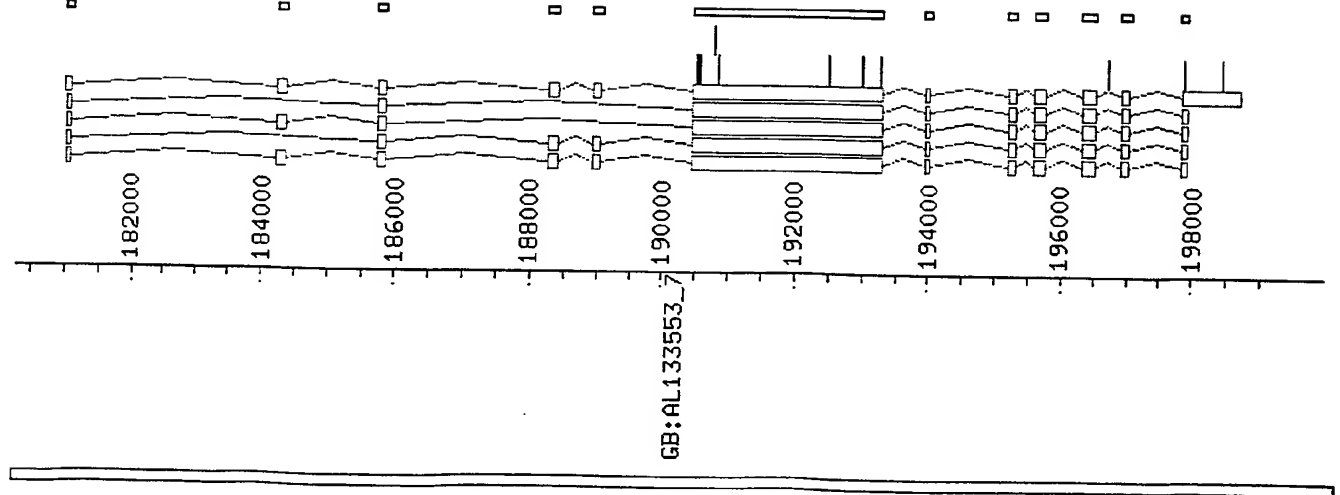


FIGURE 2

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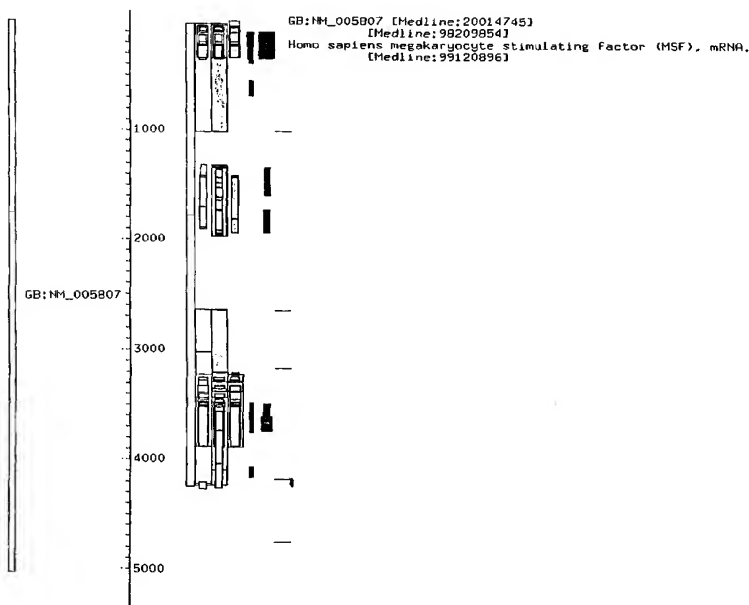
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(54) Title: NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH OSTEOARTHRITIS



(57) Abstract: The invention relates to novel polynucleotides associated with human disease, and in particular to osteoarthritis. The invention further relates to polymorphic polynucleotides associated with osteoarthritis. The invention provides methods of determining if a particular polymorphism predisposes an individual to or is associated with the development of osteoarthritis. The invention also provides methods of detecting the presence of one or more polymorphism as an indicator of osteoarthritis, and provides for use of novel polynucleotides of the invention in the development of drugs and in disease treatment.

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/41225

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : C12Q 1/68; G01N 33/00, 33/53; C12P 19/34; C07H 21/04; C12N 1/21, 5/10, 15/63; A01K 67/00; C07K 14/00  
US CL : 435/6, 7.1, 91.2, 252.3, 320.1, 325, 810; 536/23.5, 24.31, 24.33; 530/350; 800/3, 8

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 91.2, 252.3, 320.1, 325, 810; 536/23.5, 24.31, 24.33; 530/350; 800/3, 8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Continuation Sheet

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THUR et al. Mutations in cartilage oligomeric matrix protein causing pseudoachondroplasia and multiple epiphyseal dysplasia affect binding of calcium and collagen I, II, and IX. The Journal of Biological Chemistry. 02 March 2001, Vol. 276, No. 9, pages 6083-6092, see entire reference, particularly pages 6083-6084.	11-15, 21-23, 25 -----
Y	US 6,265,157 B1 (PROCKOP et al) 24 July 2001 (24.07.2001), see entire reference, particularly col 6, line 54-col 10, line 62; col 27, lines 20-38.	1-10, 16-20, 24, 26-33
Y	AHERN, H. Biochemical, reagent kits offer scientists good return on investment. The Scientist. July 1995, Vol. 9, No. 15, pages1-5, see entire reference, particularly page 4/5.	1-10, 16-20, 24, 26-33
Y		29, 30, 33



Further documents are listed in the continuation of Box C.



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"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;"

document member of the same patent family

Date of the actual completion of the international search

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# INTERNATIONAL SEARCH REPORT

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## Continuation of B. FIELDS SEARCHED Item 3:

USPT, CAPLUS, PGPUB, MEDLINE, LIFESCI, SCISEARCH, EMBASE, BIOSIS, CAPLUS

search terms: bone morphogenic protein 2, bone morphogenetic protein 2, bmp2, cartilage intermediate layer protein, cilp, cartilage oligomeric matrix protein, cartilage matrix protein, cartilage matrix glycoprotein, tissue inhibitor of metalloproteinase 1, timp1, tetranectin, matrix metalloproteinase 3, mmp3, transin, stromelysin, prostaglandin endoperoxide synthase 2, cyclooxygenase 2, cox2, osteoarthritis, joint, multiple epiphyseal dysplasia, polymorphism, mutation, osteophyte; inventors' names